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# COMPARISON OF UV SPECTROPHOTOMETRY AND HPLC METHODS FOR THE DETERMINATION OF PHARMACEUTICAL EQUIVALENCE OF CLOPIDOGREL TABLET BRANDS MARKETED IN NIGERIA

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## ABSTRACT

A comparative quality control study using ultraviolet (UV) and High performance liquid chromatography (HPLC) spectroscopy was conducted to assess the pharmacopoeial quality and compliance of five brands of commercially available clopidogrel tablets marketed in some Nigerian cities. Physical examination of the brands and information such as batch number, date of manufacture and expiry, producer, country of production, label claim of drug content and registration status with the National Agency for Food and Drug Administration and Control (NAFDAC) were noted. Quality control parameters such as uniformity of weight, friability, crushing strength, total drug content, disintegration time and dissolution profile were all conducted. Results showed that the five brands were all duly registered. The brands contained clopidogrel as their active ingredient and they all met the USP specifications for the total active drug content and other control parameters such as crushing strength, friability, disintegration time and dissolution profiles. Only one brand (CP-3) failed the weight uniformity test. A comparison of the two analytical methods used showed that the HPLC method was more accurate and precise in assessing the total drug content and other related parameters and, as such, should be relied more in quality assurance analysis of drugs when compared to UV. The brands (except CP-3) were, thus, interchangeable and could be prescribed in place of one another in clinical practice.

**KEYWORDS:** Clopidogrel, Tablet, Quality Assurance, Nigeria, HPLC, UV.

## 1.0 INTRODUCTION

Clopidogrel, marketed under several brand names, is an oral drug that is used to reduce the risk of heart disease and stroke in those at high risk. It is a pro-drug which requires activation by CYP enzyme system (principally by CYP2C19) to produce its pharmacologically active metabolite.[1-3] Clopidogrel, a thienopyridine derivative, binds specifically and irreversibly to the platelet P2RY12 puringenic receptor, thereby inhibiting ADP-mediated platelet activation and aggregation.[4] Clopidogrel can be used together with aspirin in heart attacks and following the placement of a coronary artery stent (dual antiplatelet therapy).[5, 6]

Clopidogrel is chemically known as Methyl (+)-(S)-methyl-2-(2-Chlorophenyl)–2(6,7-dihydrothienol[3,2-c] pyridin-5(4H)-yl)acetate and has a molecular formula of $C_{16}H_{16}ClNO_2S$ with a molecular weight of 321.82 g/mol. It has a half-life of 7-8 hours when taken orally with an insoluble metabolite.[7]

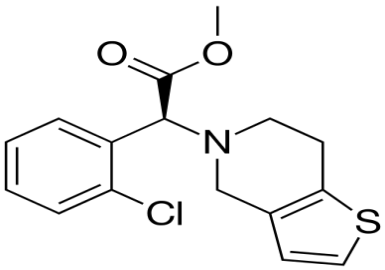


**Figure 1: Structure of (+) – Clopidogrel**

Clopidogrel is practically insoluble in water at neutral pH, but freely soluble at pH of 1, freely soluble in methanol, sparingly soluble in methylene chloride and practically insoluble in ethyl ether.

Presently, there is a multiplicity of the various brands of clopidogrel tablets in the Nigerian drug market. This, added to the high incidence of fake and counterfeit medicines in the sub-Saharan Africa, makes prescribers to be often confronted with the problem of selecting a genuine and suitable brand which is affordable and yet interchangeable with the innovator brand. According to

WHO guideline for analysis of multi-generic source of pharmaceutical products, the quality of pharmaceutical product is the most essential for efficacy and safety of the product.[8] This study, therefore, served to determine the quality of clopidogrel tablets in the Nigerian drug market as claimed on the products label, and the extent of their compliance with official specifications.

## 2. MATERIALS AND METHOD

### Sample Procurement and Assessment

**a) Samples:** The respective brands of clopidogrel tablets (encoded as CP-1, CP-2, CP-3, CP-4 & CP-5) used for this study were procured from various pharmacy premises in some Nigerian cities located at the South-South region of the country in August, 2017. Information about the various brands such as brand name, producer's name, country of manufacture, manufacturing/expiry dates, batch/or lot number, label claim of potency of the drug and product registration status with the National Agency for Food and Drug Administration and Control (NAFDAC) were assessed. The samples were also physically examined for shape, color, packaging and overall dosage form conformity.

**b) Reference Drug:** Standard clopidogrel powder was procured from Micro labs limited, India.

## METHODS

### Preparation of Simulated Intestinal Fluid (phosphate buffer), pH 7.2

This was prepared as follows: A 34 g quantity of potassium dihydrogen phosphate was dissolved in 500 ml of distilled water. The pH was adjusted to 7.2 using 0.1 N NaOH and the volume was made up to 1000 ml with distilled water.[9]

### Preparation of Simulated Gastric Fluid (SGF), pH 1.2 (without enzyme)

A 12.0 g quantity of sodium chloride was dissolved in about 5.3 L of distilled water and the pH adjusted to 1.2 using 0.1 N concentrated hydrochloric acid. The volume was made up to 6.0 L.[9]

### Weight Variation

Twenty (20) tablets were selected randomly and weighed individually. The average weight was calculated and individual weight was compared to the average weight. The tablet batches pass the test if not more than two of the individual weights deviate from the average weight by more than ± 7.5% and none deviated by twice ± 7.5%/.[10]

### Crushing Strength

Ten tablets were randomly selected from each brand of clopidogrel. The tablet crushing strength was determined using Monsanto tablet hardness tester (Monsanto, India).[10]

### Friability Test

The percentage friability of the tablets from each brand was determined using Erweka® friabilator. It should be less than 1%. Ten tablets taken from each brand were selected randomly and weighed, then placed in the friability test apparatus and rotated about 100 times. The tablets were then carefully dusted and reweighed to ascertain weight loss.[10]

### Disintegration Test

The disintegration test was performed according to pharmacopoeial procedure. Six tablets from each formulation were weighed and placed in the baskets. The apparatus (Erweka® ZT122) was operated using SGF, pH 1.2 as immersion fluid at 37± 1°C for 2 h. The tablets were observed for any sign of disintegration, cracking or softening. The tablets were then removed and the immersion fluid replaced with SIF (phosphate buffer; pH 7.2). The apparatus was operated on same condition as SGF for 1 h.[10]

### Dissolution Test

Drug release studies were carried out using an Erweka® DT600 dissolution test apparatus set at 100 rpm for 1 h in simulated gastric fluid (pH 1.2), and after that, for 1h in intestinal fluid (phosphate buffer, pH 7.2) as dissolution medium at 37°C ± 1°C. After an interval of 10, 20, 30, 40, 50 and 60 min respectively, 10 ml of the samples were taken out and 10 ml of fresh phosphate buffer pH 7.2 added to keep the volume of dissolution medium constant. The sample was analyzed using UV spectrophotometer at 240 nm for both simulated gastric fluid and simulated intestinal fluid and the percent drug release was calculated.[10]

### Content of Active Ingredient Using Ultraviolet (UV) Method

Ten tablets/capsules from each brand of clopidogrel were crushed to powder in a mortar (or poured out of the capsules). A 10-mg equivalent of clopidogrel was weighed, transferred into a volumetric flask and dissolved in 100 ml of phosphate buffer. The solution was filtered through a Whatman® filter paper. A 2 ml volume of the filtrate was withdrawn and diluted to 10 ml. The absorbance of the resulting solution was measured at the 240 nm against a solvent blank using a Labtech® UV/Vis Spectrophotometer. The mean percentage drug content was determined for each brand.[10]

### High Performance Liquid Chromatography (HPLC) Analysis of Drug Content

A Hitechilachrom elite HPLC system was used which had the following column details (Material: Chomega ZECP C-18, Particle Size: 5u, Pore Size: 100 Å, Dimension: 25 cm X 4.6 mm). The mobile phase consisted of a mixture of acetonitrile-methanol-phosphate buffer (80:10:10, v/v/v). The flow rate was set at 0.9 mL/min and the detection wavelength was set at 240 nm. The test conditions included an ambient column

temperature and pressure of 60 bar or 900 psi. In preparing the sample solutions, twenty tablets each were weighed and finely powdered. A mass equivalent to 100 mg of clopidogrel was weighed from each batch and transferred into a 100 ml volumetric flask, mixed with methanol, and sonicated for 30 min. The solutions were filtered through 0.45µ filter papers. The filtrates were transferred to 100 ml volumetric flasks and diluted to marks with methanol. Aliquots of these solutions (8, 10, 12 ml) were further diluted to 100 ml with methanol to obtain solutions containing 80, 100, 120 µg/ml respectively which were injected and chromatographed.

## RESULTS AND DISCUSSION

The results of the product information and physical examination of the respective brands of clopidogrel samples used for this study are presented in Tables 1 & 2. The preliminary evaluation showed that the samples complied with basic physical assessment requirements by displaying the label claim, batch number, date of manufacture and expiration, manufacturer, country of manufacture and registration status with the National Agency for Food and Drug Administration and Control, NAFDAC in Nigeria. All the brands of clopidogrel tablets studied were duly registered and within their shelf life as at the time of the study. The samples were subjected to both qualitative and quantitative evaluation methods to assess their pharmaceutical and chemical equivalence. Qualitative evaluation included describing the tablet color, size and shape which was carried out by visual inspection.[11]

**Table 1: Product information for various brands of clopidogrel samples.**

| Sample Brand | Date of manufacture | Expiry Date | NAFDAC Registration status | Label drug content (mg) |
|---|---|---|---|---|
| **CP-1** | 02/2015 | 02/2019 | Registered | 75.00 |
| **CP-2** | O2/2017 | 01/2020 | Registered | 75.00 |
| **CP-3** | 08/2016 | 07/2019 | Registered | 75.00 |
| **CP-4** | 11/2016 | 10/2019 | Registered | 75.00 |
| **CP-5** | 09/2016 | 08/2018 | Registered | 75.00 |

**Table 2: Physical assessment of the various brands of clopidogrel samples**

| Brand Name | Colour | Shape | Packaging | Dosage form |
|---|---|---|---|---|
| **CP-1** | Pink | Round | Aluminum foil blister | Film coated tablets |
| **CP-2** | Pink | Round | Aluminum foil blister | Film coated tablets |
| **CP-3** | Red | Round | Aluminum foil blister | Film coated tablets |
| **CP-4** | Pink | Round | Aluminum foil blister | Film coated tablets |
| **CP-5** | Red | Round | Aluminum foil blister | Film coated tablets |

The results of the pharmaceutical tests for weight uniformity, disintegration, crushing strenght and friability are presented in Table 3. The United States Pharmacopoeia[9] specified that for tablets or capsules that weigh between 130-324 mg, the standard deviation of weight should not exceed 7.5% and for tablet or capsules that weigh more than 325 mg, the standard deviation should not exceed 5%.The various brands passed the test for uniformity of weight except for CP-3 which exceeded the standard deviation of 7.5%. The pharmacopeia compliance with regard to uniformity of weight of each brand studied is important since the uniformity of dosage unit can be demonstrated by either weight variation or content uniformity study.[12] These either reflect indirectly or measure directly the amount of drug substance in the tablet.[13]

The tablets of various brands studied disintegrated between 15.66 ± 0.23 - 24.43 ± 0.28 minutes. All the tablet brands passed the disintegration test of less than 30 minutes for film-coated tablets.[10] The USP recommends a crushing strength of 4 - 5 kp for coated tablets. The crushing strength recorded ranged from 4.27 ± 0.45 - 5.78 ± 0.65, and as such, all the brands studied passed the test for crushing strength. The percentage friability for all the samples was less than 1% as specified in official compendia.[10]

**Table 3: Results of some pharmaceutical tests of the samples.**

| Samples | Uniformity of weight (mg) | Disintegration test (mins) | Crushing strength (kgf) | Friability (%) |
|---|---|---|---|---|
| **CP-1** | 54.65±4.98 | 22.43±1.08 | 4.27±0.45 | 0.39 |
| **CP-2** | 260±1.96 | 22.45±0.31 | 4.32±0.57 | 0.42 |
| **CP-3** | 317.25±8.76 | 24.43±0.28 | 5.78±0.65 | 0.21 |
| **CP-4** | 338.85±3.65 | 15.66±0.23 | 5.75±0.60 | 0.32 |
| **CP-5** | 251.1±4.35 | 18.73 ±0.19 | 5.14±0.57 | 0.27 |

The USP specified the amount of active ingredient to fall within 90-110%. Table 4 shows the results of the actual and total percentage of the drug content for the respective brands of Clopidogrel tablets studied as against the label claim of 75 mg using both UV and HPLC for the analysis. From the UV analytical results, only CP-5 attained 100% mark for total drug content, although all the brands passed the test for total drug content.

The standard caliberation curve procedure for clopidogrel when carried out using the UV yielded the equation (1) below.

$Y= 2.314x+0.106$ ($R^2= 0.9955$)

……..(1)

Also, when the standard calibration curve was carried out on a Hitechilachrom Elite HPLC system, it yielded equation (2):

$Y=2.254-0.08X$ ($R^2 = 0.9966$)

…….(2)

For UV method, the percentage total drug content in percentage ranged between 96.00 - 104.00% while for HPLC, it ranged from 100.43 - 106.03%. According to official monographs (the USP), the drug should be equivalent to not less than 90% and not more than 110%. Thus, all the brands equally passed the test for the total drug content when analyzed with HPLC. However, it was observed that the HPLC method was more accurate and precise in assessing the quality of drugs when compared to UV analysis as the former achieved a higher percentage drug content for all the samples. It has been reported that chromatographic method (HPLC) is a more sensitive and reliable assay and the technique is usually used to further support the results by the UV.[14] UV method, however, does not require the elaborate treatment and procedures usually associated with chromatographic method.[15]

**Table 4: Comparison of the actual and percentage drug contents using UV and HPLC.**

| Brand name | Label content (mg) | UV | | HPLC | |
|---|---|---|---|---|---|
| | | Actual content (mg) | Percentage content (%) | Actual content (mg) | Percentage content (%) |
| **CP-1** | 75.00 | 74.80 | 99.73 | 75.32 | 100.43 |
| **CP-2** | 75.00 | 74.60 | 99.47 | 75.22 | 100.29 |
| **CP-3** | 75.00 | 73.00 | 97.33 | 77.30 | 103.07 |
| **CP-4** | 75.00 | 72.00 | 96.00 | 78.22 | 104.29 |
| **CP-5** | 75.00 | 78.00 | 104.00 | 79.52 | 106.03 |

The release profile of the samples at different time intervals are presented in Table 5. All the tablet brands showed a dissolution profile that met the official specification of 85% w/v dissolution at 30 minutes.[9] Variation in dissolution profile of tablets or capsules may be related to the nature of excipients used, the formulation process, the polymers used in capsule shell production or gelatin-plasticizers ratio used in capsule formulation.[16] It could also be due to the effect of heat and/ humidity in the storage of the product which may have caused cross-linking. Generally, the observed differences in drug release pattern of generic brands have been attributed to product formulation technology used by different manufacturers, which might also have to do with excipients used in the formulations.[16-18]

**Table 5: Release rates of the various Samples.**

| Samples % Release / Time(mins) | CP-1 | CP-2 | CP-3 | CP-4 | CP-5 |
|---|---|---|---|---|---|
| **5** | 64.00 | 73.00 | 60.70 | 78.00 | 68.72 |
| **10** | 71.00 | 78.00 | 72.50 | 80.45 | 75.3 |
| **15** | 78.00 | 80.00 | 82.80 | 82.50 | 86.50 |
| **20** | 85.10 | 85.00 | 87.24 | 85.00 | 87.20 |
| **25** | 89.50 | 88.00 | 90.64 | 89.00 | 88.80 |
| **30** | 93.00 | 95.00 | 92.00 | 91.50 | 92.04 |

## CONCLUSION

This quality assurance study indicated that all brands of clopidogrel tested conformed to the USP standards for drug content and other pharmaceutical tests for coated tablets. Based on the *in vitro* bioavailability tests, all the brands were considered bioequivalent and interchangeable. CP-3, however, had unacceptable weight uniformity coefficient which could likely result in erratic bioavailability profile and irregular dosage regimen. The results equally showed that the HPLC method was more accurate and precise in assessing the quality of drugs when compared to UV analysis.

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