The Physicochemical Equivalence of Eight Brands of Amlodipine Tablets in Lagos, Nigeria

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ABSTRACT
BACKGROUND: Amlodipine is a dihydropyridine calcium channel antagonist that is useful in the treatment of hypertension and angina pectoris. In addition to the innovator brand of this molecule, several generic brands are marketed in Nigeria.

OBJECTIVE: To evaluate the physicochemical equivalence of eight brands of amlodipine tablets marketed in Lagos, Nigeria.

METHODS: Physicochemical properties such as identity, weight uniformity, friability, hardness test, disintegration test, dissolution test and assay of active ingredients were performed using the methods described in the British and the United States Pharmacopoeia. Ultra-violet spectrophotometric and High performance liquid chromatographic methods were used for assay of the labelled amount of amlodipine in the products.

RESULTS: All the products passed weight uniformity and disintegration tests. Only one brand failed the friability test. Two brands had mean crushing strength less than 4kg/cm²; while only 4 brands passed dissolution test by releasing >75% of the labelled amlodipine within 45 minutes. One brand failed both assay and dissolution tests by returning less than official specifications in the general monograph for conventional tablets. In all, four of the eight sample products analyzed passed all the tests. These can be said to be physicochemically equivalent and may be clinically interchangeable or substituted.

CONCLUSION: This study further highlights the concerns over the quality of drug products marketed in a developing country. WAJM 2012; 31(3): 154–159.

Keywords: Physicochemical, equivalence, amlodipine, tablets.
INTRODUCTION

Nigeria, like many other developing countries is heavily dependent on drug importation to meet the healthcare needs of her teeming population. This has enormous implications for the quality of drugs available in the market. This concern is even greater for generic drugs which can come from wide sources; ranging from local manufacture to importation from other countries.

Amlodipine is a dihydropyridine calcium channel antagonist marketed as amlodipine besylate in 5 and 10mg tablets for oral use. The chief indications for its use are systemic hypertension and angina pectoris.1,2

Following the expiration of the manufacturer’s patent for the innovator brand some years ago, there has been an explosion of generic brands of amlodipine in Nigeria. Increased detection of hypertension as a result of more frequent community screening programmes as well as awareness campaign; and wide prescription of calcium channel antagonists among medical practitioners in Nigeria are recognised factors which have made amlodipine attractive to drug importers and marketers.3

One of the consequences is that patients and healthcare providers are exposed to a wide variety of widely-sourced brands of this drug without absolute guarantee of their efficacy. Efforts of the regulatory authority in Nigeria (NAFDAC: National Agency for Food and Drugs Administration and Control) are directed at ensuring that only products that meet set criteria and standards are registered and made available for public consumption.4 However, it is not uncommon to find unregistered drugs being marketed or batches of registered product being substandard. Regulatory activities can be augmented by inputs from researchers who conduct independent evaluation of the quality of drug products available within the public domain. The findings from such studies could impact on public health by providing guidance to policy makers and regulatory agencies.

Evaluation of the physicochemical equivalence of different brands of the same drug available for public consumption is one way of determining the quality of such drugs. This will determine whether the drugs meet certain set standards for such formulations – a measure of quality, as well as if they are bioequivalent. Thus, if they contain the same amount of active agent that will be made available to target tissues at the same rate – a measure of brand ‘switchability’ or ‘interchangeability’ will be obtained.5

The value of these independent evaluations can be inferred from findings of previous studies with similar objectives to the present study. Evidence from previous bioequivalence studies on antimalarials, antimicrobials and analgesics indicate the need for constant monitoring of different brands of the same drug to ensure quality and efficacy.6,7

While there is a great abundance of bioequivalence research across the globe, these studies appear to focus more on analgesics and antimicrobial agents. In fact, acetaminophen preparations are the most extensively studied.12–21 Thus, with the rising burden of cardiovascular and metabolic diseases, there is the need for increased focus on drugs used in the management of these conditions.

Using a combination of physical parameters, dissolution test, and chemical assay; this study evaluated the physicochemical equivalence of eight brands of amlodipine tablet marketed in Lagos, Nigeria.

MATERIALS

Pure amlodipine besylate powder was obtained freely from Lagos state drug quality control laboratory in Lagos, Nigeria; while 8 brands of amlodipine 5mg tablets were procured from registered pharmacy stores in the city. All the tablets for each brand were from the same batch. Reagents used for the experiments included: 0.1N hydrochloric acid and HPLC grade acetonitrile (Sigma Aldrich, Germany) and methanol (Sigma Alrich, Germany).

METHODS

Uniformity of Weight

Using the Mettler-Toledo® weighing boat, 20 tablets of each brand of 5mg amlodipine were first weighed together to obtain a total weight and average weights were determined. Each of the 20 tablets was then weighed individually. The deviation and percentage deviation of each tablet from the mean weight were calculated.

Friability Test

Twenty tablets of each brand were weighed together and placed inside the Roche® friabilator which made 25 revolutions per minute for 4 minutes; making a total of 100 revolutions. The tablets were then removed, dusted, and weighed together again. The weight difference and the percentage friability were calculated for each brand.

Test of Hardness

Twenty tablets from each brand were tested for crushing strength in the Schleuniger-2E hardness tester. Each tablet was placed diametrically on the testing rail and the machine was then switched on. The tablet was crushed by horizontally sliding blocks which stopped moving on meeting. The crushing force (Kg) was read on the indicator.

The crushing force for each tablet was recorded and the average for the 20 tablets of each brand was calculated.

Disintegration Test

For each of the eight brands of the 5mg amlodipine, 6 tablets were evaluated for rate of disintegration using the Manesty® Tablet Disintegration Test Unit. Each tablet was placed in each of the 6 basket tubes making up the unit. Simple harmonic vertical motion raised and lowered the tubes into the water bath. The bath temperature was maintained at 37±0.5°C. The time taken for each tablet to go into solution leaving no residue was recorded indicating the end point.

Dissolution

This was carried out using the Electrolab® Dissolution Tester (ETC 0702067), which features the paddle method and eight dissolution vessels bathed in a water bath whose temperature was always maintained at 37±0.5°C, with paddles programmed to make 50 revolutions per minute.

Each vessel was cleaned with distilled water, rinsed with 0.1N hydrochloric acid (0.1N HCL), wrapped with aluminum foil paper to exclude light rays (to prevent photolysis of the light-
sensitive amlodipine), and filled with 900mls of 0.1N HCL. Six tablets from each brand were evaluated. Each was placed in a dissolution vessel and the system was powered.

Samples (5mls) were obtained at 5, 10, 15, 20, 30, 40, and 60 minutes. 5mls 0.1N HCL blank was returned to the vessel each time a sample was taken. Each 5ml sample was filtered with a Millipore syringe filter (0.45µm) while being transferred into a labeled 5mls sample bottle which was also wrapped with aluminum foil paper. Analysis of the dissolution samples was carried out immediately using validated Ultraviolet spectrophotometric method.

**Standard Calibration Using UV and HPLC**

Amlodipine reference standard (5mg) was accurately weighed, dissolved in 0.1 N HCL and made up to 5mls to obtain 1000µg/ml. Gradient concentrations (2.5, 5, 7.5, 10, 15, 20, 40 µg/ml) were prepared from stock solution. The filtered dissolution samples were diluted 1 in 100. Each sample was put in the spectrophotometer, and absorbance was taken at 238nm.

Gradient concentrations (10–100µg/ml) amlodipine standard were also analyzed using HPLC with the chromatographic conditions stated below. Results obtained were used to plot calibration curves of amlodipine standard.

The intra and inter-day assay precision was carried out by estimating the corresponding responses three times on the same day and on three different days for three different concentrations.

**Chemical Assay**

Chemical assay was conducted on all the brands using both UV spectrophotometry and High Performance Liquid Chromatography (HPLC). This was done to determine the actual amount of amlodipine present in the 5mg tablet formulation.

**Sample Preparation for UV Spectrophotometry**

Twenty tablets from each brand were weighed to determine the average weight. The tablets were then triturated in a porcelain mortar into fine powder. The equivalence of 5mg of amlodipine was weighed out and transferred into a 5ml sample bottle. This was dissolved and made up to 5mls solution with 0.1N HCL, to obtain 1000µg/ml stock solution. Six replicates, 40µg/ml solution was prepared for each brand. This was then sonicated in ultrasonic bath and filtered with syringe filter (0.45µm). The absorbance of the filtrates were read in UV/visible spectrophotometer and the concentrations were determined from the calibration plot of the standard.

**Sample Preparation for HPLC**

The equivalence of 5mg of amlodipine was weighed out of the triturated fine powder and transferred into a 5ml sample bottle. This was dissolved and made up to 5mls solution with 0.1N HCL, to obtain 1000µg/ml stock solution. Six replicates, 40µg/ml solution was prepared for each brand from various stock solutions of the tablets. They were then sonicated in ultrasonic bath and filtered with Acrodisc® syringe filter (0.45µm). The filtrate was injected into the HPLC. Each concentration was evaluated from the calibration plot of the standard.

**Chromatographic Conditions For HPLC**

The HPLC system was performed with an Agilent® Liquid Chromatograph equipped with Quartenary pumps, a degasser, UV absorbance detector and a Rheodyne injection valve supplied with a 20µl loop. The analytical column was a stainless steel Zorbax XDB® 150 x 4.6mm I.D, 5µm particle size. Temperature of the column was maintained at 35°C and the flow rate was 0.8ml/minute with 20µl injection while the detection wavelength was 238nm.

**Data Analysis**

Analytical data obtained from the experiments were collected on data sheets and entered into Microsoft Excel 2005 (Microsoft Inc, Denver, Colorado, USA.) for validation and storage. Statistical analysis was carried out using SPSS (Statistical Package for Social Sciences) version 14 for Windows (SPSS Inc. Rochester, MN, USA.) and Microcal data analysis and Technical Graphic Origin® version 6.0 software (Northampton, MA, USA).

**RESULTS**

The description of each brand is provided in Table 1. Brand D had no manufacturing date on the pack and also was not registered by NAFDAC. Table 2 shows the physical properties of the evaluated brands. At the time of evaluation, all the products were still within their shelf lives. All the 8 brands passed uniformity of weight as none of the brands deviated by up to 10% from the mean value as specified by the British Pharmacopoeia (B.P.). Only brand F had a weight loss of >1% following friabilization. The United States Pharmacopoeia (USP) states that a satisfactory tablet should have a weight loss of not more than 1% after friabilization. Two brands, C

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### Table 1: Brand Designation and Coding

<table>
<thead>
<tr>
<th>Brand Code</th>
<th>Batch Number</th>
<th>Manufacturing Date</th>
<th>Expiry Date</th>
<th>NAFDAC Registration Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>910039634</td>
<td>February 2009</td>
<td>January 2013</td>
<td>04–1386</td>
</tr>
<tr>
<td>B</td>
<td>01B0925</td>
<td>November 2009</td>
<td>October 2011</td>
<td>04–9891</td>
</tr>
<tr>
<td>C</td>
<td>A05801</td>
<td>November 2008</td>
<td>October 2012</td>
<td>A4–2112</td>
</tr>
<tr>
<td>D</td>
<td>C81924</td>
<td>Not Provided</td>
<td>April 2010</td>
<td>Not Provided</td>
</tr>
<tr>
<td>E</td>
<td>AMG 005</td>
<td>November 2007</td>
<td>April 2010</td>
<td>A4–0441</td>
</tr>
<tr>
<td>F</td>
<td>90105100</td>
<td>August 2009</td>
<td>August 2012</td>
<td>A4–0332</td>
</tr>
<tr>
<td>G</td>
<td>0005</td>
<td>June 2008</td>
<td>June 2011</td>
<td>A4–0580</td>
</tr>
<tr>
<td>H</td>
<td>12619</td>
<td>April 2009</td>
<td>March 2011</td>
<td>A4–1804</td>
</tr>
</tbody>
</table>
Physicochemical Equivalence of Amlodipine

and H had mean crushing force of <4Kg/cm², these brands failed the hardness test. However, all the eight brands passed the disintegration test; all 8 disintegrated within the 15 minutes required by the B.P.²²

Calibration plots were generated for the assays of standard amlodipine using UV spectrophotometer and HPLC. The linear regression equations were \( y = 0.0182x + 0.004 \) and \( y = 14.919x – 99.785 \) with correlation coefficient of 0.999 and 0.992 in UV and HPLC respectively. The intra and inter day assay precision of samples expressed as coefficient of variation (CV) gave value < 2% for both analytical procedures.

Figure 1 illustrates the dissolution profiles of the 8 brands. Brand C, D, G and H were able to release 75% of the labeled amount of amlodipine within 45 minutes as required by the general requirement for convention tablets.²² The average percent purity of the labelled content, amlodipine evaluated from each brand using UV spectrophotometer and HPLC are shown in Table 3.

DISCUSSION

We have evaluated the physicochemical equivalence of 8 brands of amlodipine tablets sourced from different pharmacy stores in Lagos, Nigeria. Our findings indicate four of the brands passed all the tests conducted and these products can be described as pharmaceutical equivalent. One of the brands can be said to have failed the assay test by having percent purity below 90-110% which is the general requirement for tablets formulation as stipulated by official book.²² Though there is no pharmacopeial monograph yet for amlodipine formulation in the official books, the four brands that made the 75% release suggest that these were the only brands whose formulation allowed the release of the required amount of the active substance within the specified time. This may have implications for the in-vivo pharmacokinetics of the brands studied. Brand A appeared to be a substandard product since the amount of amlodipine detected by both assay methods were far below the minimum

Table 2: Brand Evaluation by Physical Parameters

<table>
<thead>
<tr>
<th>Brand</th>
<th>Uniformity of Weight (Mean % Deviation ± SEM)</th>
<th>Friability (% Change in Weight after Friabillation)</th>
<th>Hardness: Mean Crushing Force (Kg/m²)</th>
<th>Disintegration Time (Seconds)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.996 ± 0.187</td>
<td>0.025</td>
<td>10.85 ± 1.03</td>
<td>20</td>
</tr>
<tr>
<td>B</td>
<td>1.303 ± 0.398</td>
<td>0</td>
<td>5.14 ± 0.59</td>
<td>33</td>
</tr>
<tr>
<td>C</td>
<td>1.674 ± 0.224</td>
<td>0.699</td>
<td>1.99 ± 0.64</td>
<td>136</td>
</tr>
<tr>
<td>D</td>
<td>0.685 ± 0.098</td>
<td>0.214</td>
<td>6.49 ± 0.33</td>
<td>8</td>
</tr>
<tr>
<td>E</td>
<td>1.421 ± 0.185</td>
<td>0.286</td>
<td>4.75 ± 0.60</td>
<td>7</td>
</tr>
<tr>
<td>F</td>
<td>1.514 ± 0.221</td>
<td>1.277</td>
<td>5.83 ± 0.90</td>
<td>9</td>
</tr>
<tr>
<td>G</td>
<td>2.967 ± 0.732</td>
<td>0.206</td>
<td>5.08 ± 1.41</td>
<td>36</td>
</tr>
<tr>
<td>H</td>
<td>1.041 ± 0.156</td>
<td>0.40</td>
<td>2.75 ± 1.07</td>
<td>4</td>
</tr>
</tbody>
</table>

* SEM: Standard error of mean.

Table 3: Chemical Assay of Amlodipine Tablets using Ultra-Violet Spectrophotometry and High Performance Liquid Chromatography (HPLC)

<table>
<thead>
<tr>
<th>Brand</th>
<th>% Detected (using UV Spec.)</th>
<th>% Detected (using HPLC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>66.3 ± 1.1</td>
<td>46.0±2.1</td>
</tr>
<tr>
<td>B</td>
<td>101.8 ± 2.3</td>
<td>106.8±1.9</td>
</tr>
<tr>
<td>C</td>
<td>96.4 ± 1.6</td>
<td>99.5±2.2</td>
</tr>
<tr>
<td>D</td>
<td>105.8 ± 1.8</td>
<td>100.4±3.1</td>
</tr>
<tr>
<td>E</td>
<td>107.9 ± 2.4</td>
<td>108.5±1.6</td>
</tr>
<tr>
<td>F</td>
<td>109.7 ± 2.9</td>
<td>107.8±2.1</td>
</tr>
<tr>
<td>G</td>
<td>106.2 ± 1.6</td>
<td>101.3±1.2</td>
</tr>
<tr>
<td>H</td>
<td>100.3 ± 1.3</td>
<td>102.3±1.9</td>
</tr>
</tbody>
</table>

Fig. 1: Dissolution Profiles of the 8 Brands of Amlodipine Tablets

expected by specifications likewise its in-vitro release was also below approved general release requirement.

A similar study which evaluated ten brands of ciprofloxacin tablets in this environment identified three registered brands that had relatively low content of active substance.\(^2\) A study on 85 generic products from 21 countries reported that 91% of the generic piroxicam products evaluated failed to meet the routine in-vitro USP quality assurance criteria for potency and or dissolution.\(^3\) Although all the brands investigated passed the uniformity of weight and disintegration tests. These two physical parameters were insufficient to confer physico-chemical equivalence on the 8 brands. Only four brands of the eight studied could be said to be pharmaceutically equivalent hence may be interchanged clinically. Furthermore, 3 brands did not meet the requirements for two other physical parameters evaluated: F was more friable than the others while C and H had low crushing strengths.

Conclusion
This physicochemical evaluation showed that four out of the eight brands widely sourced amlodipine tablets in this environment could not meet the entire pharmacopoeial standards; and also exhibited variations in individual properties that would make inter-brand substitution unjustifiable. This underscores the need for continuous monitoring of the safety, quality and efficacy of essential drugs in this environment.

ACKNOWLEDGEMENT
The authors express their gratitude to the management and staff of SKG Pharma, Lagos, Nigeria; for allowing access to their laboratory for the evaluation of the samples’ physical parameters.

Duality of Interest
The authors declare that they have no conflict of interest that could affect the conduct or the findings of this study. We also wish to state that SKG Pharma was not involved in data collection, data analysis or the preparation of this publication. At the time the study was conducted the company was neither manufacturing nor marketing amlodipine tablets.

REFERENCES