Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 7, Issue 10, 2015

Original Article

EVALUATION OF THE PHYSICOCHEMICAL PROPERTIES AND QUALITY INDICES OF MULTISOURCED 5 MG AMLODIPINE BESYLATE MARKETED IN SOUTHERN NIGERIA

ERAGA SYLVESTER OKHUELEGBE*, ORAKWE CLIFFORD AMARA, IWUAGWU MAGNUS AMARA

Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy, University of Benin, Benin City, 300001, Nigeria
Email: eragaso@uniben.edu

Received: 14 Apr 2015 Revised and Accepted: 08 Aug 2015

ABSTRACT

Objectives: This study was carried to evaluate and compare the physicochemical parameters and cost of available brands of amlodipine besylate 5 mg marketed in Southern Nigeria.

Methods: Fifteen brands were subjected to weight uniformity, friability, hardness, disintegration time, dissolution and chemical content tests. The chemical content test was performed using RP-HPLC method with isocratic run using acetonitrile: acetone buffer (50:50) as mobile phase at a flow rate of 0.8 ml/min and 237 nm wavelength of detection.

Results: All the brands tested passed the weight uniformity test with no significant difference in values within each brand at $p<0.05$. The crushing strength values of only five brands were within official specification. All the brands passed the disintegration time and friability tests while only ten brands passed the content assay. The dissolution test revealed that all the brands released 70 % of their drug content within 45 min. The shelf price of the innovator product was N8, 500 (USD 43) for a pack of 100 tablets while the other products were about N3,000 (USD 15) for an equivalent pack.

Conclusion: It can be concluded that though all the brands tested showed good dissolution profiles, only ten brands could be regarded as pharmaceutical equivalents according to their content assay and the price disparity between the products studied cannot be justified by the outcome of this physicochemical evaluation.

Keywords: Amlodipine, 5 mg, Multisource products, Physicochemical evaluation, RP-HPLC.

INTRODUCTION

The goal of antihypertensive therapy is to abolish the risks associated with blood pressure elevation without adversely affecting quality of life. Drug selection is based on efficacy in lowering blood pressure and in reducing cardiovascular end points including stroke, myocardial infarction and heart failure [1]. Amlodipine is well tolerated by majority of patients with very limited side effects. Its prolonged half-life ($t_{1/2}$) of 35-50 h when administered at a dose of 10 mg daily offers maximum convenience to the patient [2].

Medication cost is a chief determinant of the affordability and adherence to any treatment regimen. The high cost of branded products has encouraged the influx of generics (unbranded drug products) into Nigeria which are cheaper and are expected to be bio-equivalent to the branded. The essential drug concept supports the use of generic medicines so as to improve access to essential medicines via drug price control. A generic medicine is defined as an exact simulation of an established drug product (called the branded product), not protected by a patent and promoted with the chemical name of the active ingredient [4].

The influx of so many generic products into the country has led to reports of substandard and counterfeit drugs which are often less expensive in order to attract higher market patronage [5]. Also the emergence of newer generics in the market and the disappearance of older ones have created some alarming fears and worries on the minds of the patients (the end users) and stakeholders over the years. Quality control tests are important tools that can be used in assessing the genuineness of drug products before their consideration for possible substitution and/or inter changeability of different multi-source brands of a drug [6].

For instance, a study was carried out in 2011 that evaluated ten brands of amlodipine tablets sourced from some southern states of Nigeria [7]. A similar study carried out two years later identified three new products in circulation in the same region [8]. The need to continuously assess the equivalence of clinically useful pharmaceutical multi brands and generics cannot be over emphasized.

HPLC is considered the best technique for developing precise, accurate, linear, robust, stable and rugged analytical methods in pharmaceutical dosage forms [9]. Speed and accuracy are the most crucial aspects in quality control laboratories and HPLC analyses afford both advantages. European Pharmacopoeia describes assay of amlodipine besylate by reversed phase high performance liquid chromatography in bulk and pharmaceutical formulations. Amlodipine besylate behaviour and quality had been studied extensively by methods such as spectrophotometric [10], voltametric [11] and chromatographic (12].

This study is geared towards ascertaining the credibility of the labelled claims of the available brands of amlodipine besylate sold in ten southern states of Nigeria. It is also designed to determine whether the price differences between these numerous generics of amlodipine in the market are a reflection of the quality of the products.

MATERIALS AND METHODS

The samples (table 1) studied were purchased from pharmacies across ten southern States in Nigeria (namely: Lagos, Ondo, Edo, Delta, Anambra, Enugu, Imo, Ebonyi, Rivers and Akwa-Ibom). Available brands in each state were selected to make up the fifteen brands studied. Brands already selected from one state were no longer considered in any other state. Pure amlodipine powder was gratefully received from Juhel Nigeria Limited, Enugu State, Nigeria. All other reagents were of analytical grade and water was double distilled.

Weight uniformity

The weight of each of 20 tablets was determined from each brand using an electronic balance (College B154, Mettler Toledo, Switzerland) and the mean weight and standard deviation were computed.

Hardness

The crushing strength of ten tablets per brand was determined by diametric compression of each tablet (Campbell Electronics, Model HT-30/50, India). The mean value was calculated.

Friability

Ten previously weighed tablets per brand were placed in the drum of a friabilator (Erweka GmbH, Germany) revolving at 25 rpm. After

4 min, the tablets were brought out, dedusted and reweighed. Friability was calculated as the percentage loss in weight.

Disintegration time

The disintegration times of six tablets per brand were determined in distilled water at 37±0.5 °C using the BP disintegration tester (MK IV, Manesty Machines, UK).

Content of active drug

Chromatographic conditions

Chromatographic separation was performed on an Agilent 1200 Infinity Series (Agilent Technologies Inc., USA) arranged with a gradient pump, rheodyne injector, column oven and VWD detector. An Agilent ZORBAX XDB, 150 mm x 4.6 mm, 5-µ column was used as the stationary phase. Drug samples were separated isocratically with a mobile phase consisting of acetonitrile and 0.025 M potassium dihydrogen phosphate buffer adjusted to pH of 4.0±0.1 (60:40) at a flow rate of 0.8 ml/min. The analysis was carried out at 30 °C and the injection volume was 10 µl. The detector was set at 237 nm.

Mobile phase preparation

Potassium dihydrogen phosphate salt (4 g) was weighed into a 1 L beaker and dissolved with sufficient distilled water. Triethylamine (0.5 ml) was added and the pH was adjusted to 4.0 with ortho-phosphoric acid. The volume was then adjusted to 1 L. Four hundred millilitres of the solution was transferred into a beaker and 600 ml of HPLC grade acetonitrile was added to it. The premix mobile phase was filtered through a 0.45 µm nylon filter with the aid of a vacuum pump and then used in equilibrating the HPLC column and system.

Standard calibration curve

The standard solution of amlodipine was prepared by weighing amlodipine besylate working standard equivalent to 5 mg and diluting with 10 ml of methanol-distilled water mixture (50:50). From the stock solution, serial dilutions were made to obtain solutions of concentrations ranging from 5-100 µg/ml. Six injections of the final solutions were run on the HPLC system to determine system suitability and also calibrated to quantify the samples. The mean peak area (mPA) of the determinations for each concentration was plotted against the respective concentration to get the calibration curve.

Sample preparation

Twenty tablets of each brand were weighed and pulverized into powder. A quantity of the powder equivalent to 5 mg of amlodipine besylate was weighed into a 100 ml volumetric flask and dissolved with about 70 ml of the diluent (methanol: distilled water). The sample was sonicated for about 10 min and then made up to 100 ml with sufficient diluent. A 1 ml aliquot of the solution was further diluted to 100 ml and filtered before injection into the chromatographic system [13]. Three injections were run on each brand and the average peak area for the triplicate measurement was extrapolated on the calibration curve derived from the pure amlodipine powder to obtain the equivalent concentration and the percentage content calculated [14]. The mobile phase was also run as the blank.

Dissolution studies

Various amlodipine standard concentrations ranging from 1.0-25 µg/ml were prepared from stock solutions with 0.1M HCl and subjected to ultra-violet spectrophotometric analysis at 237 nm (T70, PG Instruments Ltd, USA). Respective absorbances were taken and lines of regression were determined. The calibrator prepared was used for the analysis of the dissolution samples.

Dissolution tests were carried out using a BP dissolution test apparatus (Caleva ST7, GB Caleva. UK). This was fitted with a basket rotated at 100 rpm using 900 ml of 0.1M HCl (pH 1.15) solution as the dissolution medium maintained at 37±0.5 °C. Three tablets selected at random from each brand were used simultaneously for the study. A 5 ml volume of dissolution fluid were withdrawn at various intervals and replaced with an equivalent volume maintained at the same temperature (37±0.5 °C). This was continued for 60 min. The sample was filtered and diluted with an equal volume of 0.1M HCl. The absorbances of the resulting solutions were measured at λmax of 237 nm. The percentage of drug released was then calculated from the equation obtained from the calibration curve.

Statistical analysis

All the results obtained are expressed as mean±standard deviation and were subjected to the student t-test statistical analysis to test for significance of difference using Microsoft Excel 2007. $p<0.05$ was considered to be statistically significant.

RESULTS AND DISCUSSION

All the fifteen (15) brands of amlodipine used in the study were uncoated immediate release tablet dosage forms and were still within their shelf life as at the time of study. All except one were registered with National Agency for Food Drug Administration and Control (NAFDAC). The summary of the drug codes and details including the estimated prices in the region are presented in table 1. All the brands were packaged in photo-protective foil strips to prevent light degradation. When observed visually, all the brands showed similarities in colour as they were mostly white with a slight difference in size, shapes and inscriptions.

The results of the weight uniformity test are shown in the table 2. All the brands complied with weight uniformity test specifications which stipulate that not more than two tablets should have maximum deviation from the mean weight of 5 % and none with a maximum deviation of 10 % [14]. This indicates that the individual doses are likely to be dependable and consistent with each repetitive intake.

The results of the hardness test are shown in table 2. Adequate tablet hardness is essential to ensure resistance to damage during handling, packaging and transportation. Brands A2, A8 and A13 were well within the acceptable limit of 5.0-8.0 kp [14] although crushing strength values as low as 4.0 kp is considered minimum for a satisfactory tablet [15].

Brands A3, A9 and A10 were below the acceptable limit of hardness. This may account for the brittle nature of brand A3, as some of the tablets crumbled on being removed from their blister pack. Also, A1, A4-A7, A14 and A15 were above the acceptable limit. This may be attributed to excessive compression pressure during tableting or too much binder. Care should be taken by manufacturers not to formulate an overly hard tablet as it may adversely affect disintegration time and in turn disso lution.

All the brands under investigation passed the friability test as shown in table 2. They exhibited friability values ranging from 0.03 % to 0.69 % which is well below the stipulated maximum permissible value of 1 % loss of weight of the tablet tested [14]. This test also indicates the ability of the tablets to withstand wear during handling and transportation. This alongside hardness is an indication of appropriate choice of binder and compression pressure.

All the brands disintegrated within 15 min (table 2). This is well within the acceptable limit for uncoated immediate release tablets. Brand A9 showed the least disintegration time of 5 sec while brand A7 showed the highest value of 548 seconds (9.13 min).

The results of the chemical content assay are also shown in table 2. Only brands A1, A7 and A8 had chemical contents below the official specification of 95 % to 105 % as stipulated in BP, 2011 [16]. The innovator product (Br and A1) had a chemical content slightly below the official standard. This may be as a result of non-compliance with GMP, production negligence or drug instabilities. Amlodipine is known to be thermo-labile, therefore poor storage conditions probably employed by the intermediaries between the manufacturers and patients may have caused degradation which may account for the low chemical content observed in these brands. Products A2 and A6 showed high chemical content above the permitted official value. This may be due to production error. However, the fact that out of the fifteen brands assessed, only nine passed the content assay is worrisome. This is seriously implicated in numerous drug therapy failures observed in Nigeria today [17].

Table 1: Selected brands of amlodipine besylate (5 mg) tablets used in the study

Brand code	Country of origin	Manufacture date/expiry	Batch/ Lot No.	NAFDAC No.	Pack size	Price (N)	Brand name
A1	Pfizer Manufacturing GMBH, Germany	07/2013 06/2017	D10269031	04-1386	10×10	8500	Norvasc
A2	Atlanta Pharma Sintral, Portugal	01/2014 01/2016	KA0045A	A4-0908	10×3	1200	Lofral
A3	Neimeth International Pharm. PLC, Nigeria	02/2014 02/2017	40105100	A4-0332	10×10	4500	Amlovar
A4	Baroque Pharmaceuticals Pvt Ltd, India	05/2013 04/2016	(10)B3021	A4-3433	10×3	450	Ambes
A5	Zhejiang Pharmaceutical Co Ltd, China	05/2013 04/2015	130501	A4-4070	10×3	500	Cladipine
A6	Macleods Pharmaceuticals Ltd, India	04/2013 03/2016	EAY301A	A4-6991	10×3	350	Amland
A7	Zhejiang Pharmaceutical Co Ltd, China	09/2013 08/2016	130903	A4-4131	10×3	500	Pemaload
A8	Jiangsu Ruinian Qianjon Pharm. Co Ltd, China	07/2013 07/2016	130713	A4-7894	10×3	400	Rodivas
A9	MA Holder Teva UK Ltd, UK	07/2014 06/2019	4G73UK	-	14×2	400	Amlodipine
A10	Maxheal Laboratories Pvt Ltd, India	03/2013 02/2016	NP3003	A4-8407	10×3	750	Norapine
A11	Divine Essentials Formulations, Nigeria	06/2012 05/2015	015AS	04-3149	10×3	750	Amlovas
A12	Strides Vital Nig. Ltd, Ikeja, Nigeria	08/2013 07/2016	5640281	A4-4593	10×10	1800	Amloster
A13	Mercury Laboratories Pvt Ltd, India	08/2014 07/2017	14160002	A4-1664	10×3	600	Amlocard
A14	Juhel Nigeria Ltd Enugu, Nigeria	05/2014 04/2017	0018	A4-0580	10×10	1450	Juvasc
A15	Micro-Laboratories Limited, India	02/2014 01/2017	AMGH0014	A4-0441	10×3	400	Amlong

Table 2: Some physicochemical properties of amlodipine besylate tablets

Brand code	Weight (mg) (N=20)	Crushing strength (kp) (N=10)	Friability (%) (N=10)	Disintegration time (sec) (N=6)	Content of amlodipine (%) (N=20)
A1	200.72±1.18	10.25±0.77	0.14±0.22	14±1.50	94.92±1.50
A2	200.36±1.57	5.10±0.39	0.09±0.16	6.0±0.20	115.48±1.20
A3	201.41±3.10	2.68±0.26	0.69±0.06	9.0±0.40	98.69±1.21
A4	252.08±3.71	10.25±1.19	0.27±0.02	17±0.40	96.01±4.70
A5	154.37±2.31	10.65±0.93	0.04±0.42	290±2.32	100.15±5.21
A6	134.69±0.65	8.47±1.30	0.56±0.50	16±1.18	113.18±4.96
A7	155.92±3.04	11.42±0.50	0.40±0.62	548±5.50	91.85±6.17
A8	234.76±2.27	4.65±1.12	0.22±0.40	113±3.10	93.37±4.50
A9	202.08±1.80	3.50±1.46	0.04±0.34	5.0±1.06	100.13±1.45
A10	171.81±2.52	2.95±0.47	0.22±0.12	76±0.35	101.32±2.54
A11	307.37±4.28	6.28±1.92	0.22±0.40	60±0.60	97.74±1.55
A12	190.89±3.73	4.63±1.18	0.21±0.10	30±1.26	99.35±4.58
A13	163.50±4.61	7.35±0.67	0.24±0.20	258±3.45	95.77±4.65
A14	184.38±3.67	9.38±0.43	0.03±0.18	130±3.10	98.83±2.35
A15	191.19±1.70	8.50±0.89	0.17±0.24	11±1.60	103.37±1.50

Values are mean±standard deviation

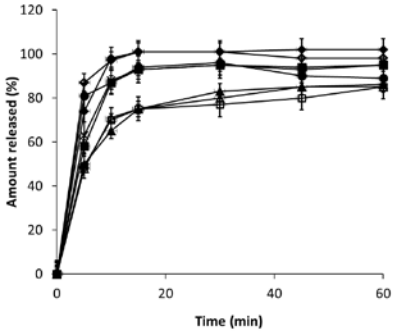

Fig. 1a: Dissolution profiles of bands A1 (◆), A2 (■), A3 (▲), A4 (●), A5 (✱), A6 (◇), A7 (□) and A8 (△). Data are expressed as the mean±SD (n= 3)

The dissolution profile plots for all the brands are shown in fig. 1a and b. All the brands showed satisfactory dissolution profiles. The BP, 2011 states that 70 % of the drug in uncoated tablet should dissolve within 45 min [16]. All the fifteen brands released more than 70 % of their contents in less than 45 min as shown in fig. 1a and b. This indicates that sufficient amount of the drug would be available for absorption to elicit the expected therapeutic effect when administered. Also, there is no significant difference ($p<0.05$) in the dissolution rates of all the brands assessed. Although comparative bioavailability studies would be required to draw clinical conclusions, the similarities obtained from the dissolution profile may indicate similarity in bioavailability and hence drug performance.

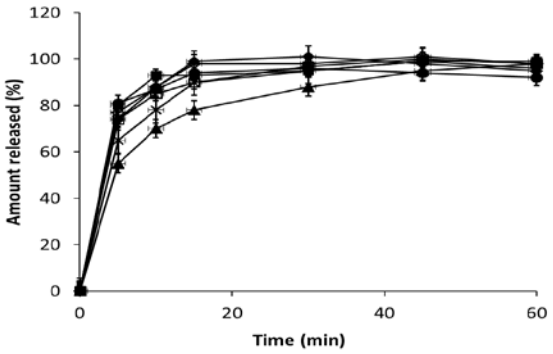

Fig. 1b: Dissolution profiles of bands A9 (◆), A10 (■), A11 (▲), A12 (●), A13 (✱), A14 (◇) and A15 (□). Data are expressed as the mean±SD (n= 3)

The prices of the brands were generally below N1,000 for a pack of 30 tablets which will amount to about N3,000 for a 100 tablets as against the innovator product sold for N 8,500. The innovator product was expected to be superior in the physicochemical and *in vitro* tests to warrant the price disparity. There was however no significant difference in the parameters tested. The wide disparity in price between the innovator product and the generics cannot be justified based on the results obtained from this study.

CONCLUSION

The failure of some of the brands tested to meet up with the labelled claim of chemical content as well as significant differences in physical parameters will require a re-evaluation on the part of the manufacturers/importers and regulating authorities to avoid allowing substandard drug products in the market. Innovator products will need to be stored and handled properly for it to continue to deliver the expected standard of actions for this may have accounted for the observed drop in the labelled claim of sample A1. The similar release profile observed among the brands may warrant inter-changeability between the brands which may not alter bioavailability significantly. The high expectation of quality action placed on the branded product resulting in high pricing may not be justified after all.

CONFLICT OF INTERESTS

No conflict of interest associated with this work

REFERENCES

1. Peikova LP, Tsvetkova BG, Kostova BD, Rachev DR. Investigations and HPLC assay of model formulations containing amlodipine besylate and lisinopril. Int J Pharm Sci Rev Res 2013;20(1, Suppl 3):11-5.
2. Katzung BG, Chatterjee K. Vasodilators and the treatment of angina pectoris. In: Katzung BG. editor. Basic and Clinical Pharmacology. 9th ed. New York: McGraw Hill Companies; 2004. p. 184-99.
3. Garattini L, Tediosi F. A comparative analysis of generics markets in five european countries. Health Policy 2000;51:149-62.
4. Osibo OO. Faking and Counterfiting of drugs. West Afr J Pharmacol 1998;12 Suppl 1:53-7.
5. Nwodo N, Uzochukwu I, Omeje E. Quality control assessment and the possibility of interchangeability between multi-sourced norfloxacin tablets marketed in Nigeria. Sci Res Essays 2007;2 Suppl 8:348-52.
6. Eichie FE, Arhewoh MI, Isesele JE, Olatunji KT. *In vitro* assessment of quality control parameters of some commercially available generics of amlodipine besylate in nigerian drug market. Int J Health Res 2011;4 Suppl 1:57-61.
7. Eraga SO, Uzochukwu OC, Iwuagwu MA. Pharmaceutical equivalence of some brands of 5 mg amlodipine besylate tablets available in southern Nigeria. West Afr J Pharmacol 2014;25 Suppl 1:38-45.
8. Aboul-Enein HY, Ali I. Chiral separations by liquid chromatography and related technologies, New York: Marcel Dekker, Inc; 2003.
9. Gohil K, Trivedi P, Molvi KI. Spectrophotometric analysis of amlodipine besylate in bulk and in tablet dosage forms. Indian J Pharm Sci 2005;67:376-8.
10. Goyal RN, Bishnoi S. Voltammetric determination of amlodipine besylate in human urine and pharmaceuticals. Bioelectrochemistry 2010;79 Suppl 2:234-40.
11. Kardile DP, Patel HH, Patel MR. Simultaneous estimation of amlodipine besylate and olmesartan medoxomil drug formulations by HPLC and UV-spectrophotometric methods. Int J Pharm Appl Sci 2011;2 Suppl 1:23-34.
12. Umapathi P, Ayyappan J, Darlin Quine S. Quantitative determination of metformin hydrochloride in tablet formulation containing croscarmellose sodium as disintegrant by HPLC and UV spectrophotometry. Trop J Pharm Res 2012;11 Suppl 1:107-16.
13. Patrick JS. Martin's Physical Pharmacy and Pharmaceutical Sciences. 5th ed. Philadelphia: Lippincott Williams and Wilkins; 2006. p. 337-54.
14. British Pharmacopoaeia. Vol. III. British Pharmacopoeia Commission. London: The Stationery Office Limited; 2009. p. 6578-85.
15. Rudnic EM, Schwartz JD. Oral solid dosage forms. In: Alfonso RG, editor. Remington: The Science and Practice of Pharmacy. 20th ed. Philadelphia: Lippincot Williams and Wilkins Inc; 2000. p. 858-93.
16. British Pharmacopoaeia. Vol. I. British Pharmacopoeia Commission. London: The Stationery Office Limited; 2011. p. 1110.
17. Olokoba AB, Obateru OA, Bojuwoye MO. Helicobacter pylori eradication therapy: a review of current trends. Niger Med J 2013;54:1-4.