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Biowaiver Study On Three Generic Brands of Amlodipine 10mg Tablets Marketed In Sudan

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Abstract

Amlodipine besylate is a BCS class I drug used in treatment of hypertension and coronary artery diseases. In this study we determined the bioequivalence of four different brands of amlodipine besylate 10 mg tablets [Norvasc (brand A), Amilodipine (brand B), Amlozal (brand C) and Amlo(brand D)] using Biowaiver, first the pharmaceutical equivalence was evaluated for the four brands using the official standards according to USP pharmacopeia including diameter, hardness, thickness, disintegration, assay and dissolution, then the biowaiver study was done according to WHO guidelines and the dissolution profiles were studied using three different buffer solutions: pH 1.2, 4.5 and 6.8, then the results were evaluated according to specifications of class 1 drugs as Amlodipine is accepted class 1. The four brands complied with requirements of the official tests of diameter, hardness, thickness, disintegration, assay and dissolution. The three brands (B, C and D) were found to be bioequivalent to innovator (Brand A) as they qualify for WHO criteria for Biowaiver (both tests and reference were very rapidly dissolving in the three medias, i.e released more than 85% in 15 minutes), so similarity factor (f2) was not needed to be calculated to evaluate the interchangeability of the four brands.

Key words: biowaiver, Amlodipine, bioequivalence, dissolution, BCS.

Introduction: Biowaiver can be defined as the acceptance for regulatory purpose for replacements of in vivo bioequivalence studies and bioavailability by in vitro dissolution when they are able to replace the in vivo dissolution reliably [1].

Biopharmaceutics classification system (BCS): is a scientific framework for classifying drug substances based on their aqueous solubility and intestinal permeability. This concept underlying the BCS finally leads to introducing the possibility of waiving in vivo bioequivalence studies in favor of specific comparative in vitro testing to product with systemic action [2]. Subsequently regulatory agencies and health organization have utilized this classification system to allow *in vitro* dissolution to be used to establish bioequivalence for highly soluble, highly permeable compounds [3].

Amlodipine besylate, is synthetic long-acting dihydropyridine calcium channel blocker. Amlodipine is used alone or in combination with other medications to treat high blood pressure (hypertension) and chest pain (angina pectoris) [4]. Amlodipine

formulations must be tested to ensure that they meet the quality assurance specifications and equivalent in their efficacy and safety.so instead of high cost and time-consuming *in vivo* study the biowaiver can be used to assess bioequivalence of different Amlodipine formulations.

Structural Formula:

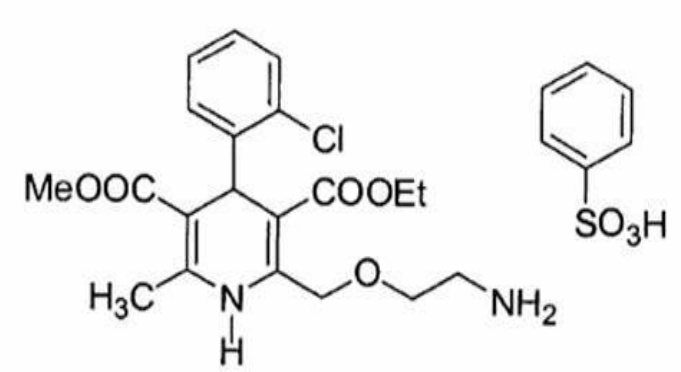

Figure (1): Chemical Structure of Amlodipine

Literature Review:

I.E Shohin, G.V Ramenskaya, G.FVasilenko, E.A Malashenko used biowaiver conditions to assess bioequivalence of some generic products of Amlodipine besylate tablets marketed in Russia, the dissolution profile of amlodipine tablets was assessed in 500mlof buffer 1.2,4.5 and 6.8using USP apparatus II, the dissolution profile of amlodipine tablets showed that both generic and innovator where rapidly dissolving (≥85% in 30 min), and so there is no need to carry out in vivo bioequivalence studies [5].

Methaq H. Sabarstudied the preparation and in-vitro evaluation of fast dissolving film of amlodipine besylate solid dispersion, since amlodipine besylate sparingly soluble orally

administered drug and the rate of absorption is often controlled by the rate of dissolution. The drug was incorporated in fast dissolving dosage form as a solid dispersion that prepared using polyethylene glycol (PEG6000) or polyvinyl pyrrolidone (PVP) in different ratios and different method of preparations. It has been that as the ratio of drug to PEG6000 or PVP In solid dispersion increased, the release rate increased and the solvent evaporation method gave grater release than fusion method. Both PEG6000 and PVP are water soluble carriers, so they lead to increase wettability and dispersibility of Amlodipine resulting in faster dissolution rate [6].

James RegunKarmoker, Prince Joydhar, ShuvroSarkar, MostaqurRahman conducted study was intended to evaluate the different physical parameters of generic amlodipine besylate tablet from different manufacturers using in vitro tests in order to minimize health risk factors and maximize the safety of local people. Six brands (A, B, C, D, E and F) of amlodipine besylate tablets (5 mg) marketed in Bangladesh were evaluated for eight in vitro tests including both official and unofficial methods. diameter test, thickness test, hardness test, friability test, uniformity of weight, disintegration test, dissolution test and assay. Dissolution study revealed brand B (99.87%) was the fastest and brand D (87.19%) was the slowest in terms of drug release. Using a validated UV spectrophotometric method assay value was recorded within 92% to 98.70%. Such a study serves as a good pointer for assessment of in vitro parameters of commercially available products which may be advantageous for future formulation development studies [7].

Justification: Amlodipine is one of important drugs prescribed. In Sudan, as in other countries a number of pharmaceutical companies market their original amlodipine formulation based on extensive clinical demand and the management strategy for stable profit. All these marketed formulations must be test to ensure that they meet the quality assurance specification and equivalent in their efficacy and safety. So instead of high cost and time-consuming *in vivo* study the biowaiver can be used to assess bioequivalent of different amlodipine formulations.

Materials and Methods

Materials

Chemicals: Working standard of Amlodipine besylate (Gift from Azal pharmaceutical industries Co. Ltd), (Hydrochloric acid and Potassium Chloride (SDFCL/ India)), (Sodium acetate, Phosphoric acid and acetonitrile (SCHARLAU/ Spain)) and (Glacial acetic acid, Monobasic Potassium Phosphate, Sodium Hydroxide,

Methanol grade and Triethylamine (DUKSAN/ Korea)).

Instruments and Equipment: Electronic balance (GR-200-EC/ UK), (Dissolution apparatus and Disintegration tester (Electro lab/ India), (pH meter (Sartorius/ Germany), (UV spectrophotometer and HPLC machine (SHIMADZU/ Japan) and Syringes, glassware (Iso lab/Germany), manual hardness tester (Monsanto/ USA) venier caliper (Qingdao/China).

Amlodipine 10mg tablets Brands: Norvasc (brand A. Innovator) 10mg (made in USA), brand B brand C and brand D (made in Sudan).

Methods

Physicochemical parameters:

The physicochemical parameters of Amlodipine10mg tablets of different brands were tested using USP methods [8] as follow:

Diameter and Thickness uniformity test

Using venier caliper diameter and hardness tests were carried out for 10 tablets of each brand. The average and the standard deviations of results were obtained.

Hardness Test

The hardness test for 10 tablets was measured using the hardness tester. The average and the standard deviations of results were obtained as shown in the table.

Disintegration Test

For disintegration test one tablet of each brand was placed in each six tubes of the basket, disk was fitted and the apparatus was operated. Water was used as immersion fluid, temperature was maintained at 37±2 ºĊ. The apparatus was operated and the time required for the 6 tablets to disintegrate was recorded.

Chemical Assay of Amlodipine tablets 10mg by HPLC

Using USP method employed to determine the drug content [9] as follow:

A-Buffer preparation: 7ml of triethylamine were added into flask of 1000 ml volume containing 900 ml of water. The solution was adjusted with phosphoric acid to pH 3.0. Then diluted with water to volume and mixed well.

B-Mobile phase: Methanol, acetonitrile, and buffer (35:15:50).

C-Standard solution preparation: 0.0275 mg/ml of USP Amlodipine Besylate RS and USP Amlodipine Related Compound A RS in mobile phase.

D-Sample solution preparation: 5 tablets from each brand were placed in suitable volumetric flask, sufficient amount of mobile phase was added to disintegrate the tablets, the mixture was shaken well for 30 minutes and diluted and filtered.

E-Procedure: Vials containing samples and standard solutions were placed in to the tray of

auto of HPLC machine. The separation was performed by column (3.9-mm*15cm; 5-µm packing L1) an ambient column temperature. The flow rate of the mobile phase was 1ml/min and isocratic system was used. Equal volume (50µL) of standard and samples were injected automatically and separately into the chromatograph, the chromatograms were recorded using UV detector 237 nm wave length. Five readings were taken for standard and three reading were taken for samples.

The quantity of Amlodipine was calculated using the following equation: Result=[peak response from sample solution / peak response from the standard solution] * [concentration of USP amlodipine besylate RS in standard solution (mg/ml)]*[nominal concentration of a peak response from the Amlodipine in the sample solution (mg/ml)]*[molecular weight of Amlodipine/molecular weight of Amlodipine besylate] * 100.

Biowaiver study

Preparation of buffered dissolution media

The medias used in the study were of pH 1.2 (hydrochloric acid solution), pH 4.5 (acetate buffer solution) and pH 6.8 (phosphate buffer solution), which were prepared using USP methods [10].

A-HCL Solution (0.1 N) pH 1.2

1.25 Liter of potassium chloride solution (0.2M) was placed in the graduated jug, 3.25 Liters of hydrochloric acid (0.2M) were added and the volume was completed to 5 Liters with distilled water and then mixed, the pH was measured during mixing until reached 1.2 value.

B-Acetate Buffer Solution pH 4.5

14.95g of sodium acetate tri-hydrate was weighed and placed in the graduated jug, 70 ml of acetic acid (2N) was added then mixed, the volume was completed to 5 Liters with distilled water and then the pH was measured during mixing until pH of 4.5 was obtained.

C-Phosphate Buffer Solution pH 6.8

1250 ml of monobasic potassium phosphate solution (0.2M) was put in graduated jug, 560 ml of sodium hydroxide (0.2M) was added and the volume was completed to 5 Liters with distilled water and then the pH measured during mixing until pH 6.8 was obtained.

Construction of Calibration Curve

Solutions of serial dilution (range from5µg /ml to 25µg/ml) of working standard of Amlodipine were prepared at each buffer solution (pH1.2, pH4.5 and pH 6.8). Then the absorbance of each concentration was determined by using UV spectrophotometer at wave length 239nm, the data obtained of absorbance were plotted against the known concentration of working standard solution at that buffer solution, three calibration curves

were drawn separately. Results are represented in figures ((2) to (4)).

Dissolution study of Amlodipine 10mg in the four brands

The dissolution profiles of Amlodipine 10 mg tablets were assessed in 500ml of buffer pH 1.2, 4.5 and 6.8 using USP apparatus II at 75rpm with teflon Paddles. In all experiments, 5ml sample aliquots were withdrawn at 5, 10, 15, 30, 45 min using syringe. All samples were filtered through 0.45-µm membrane filters. Drug release was determined spectrophotometrically by using calibration curve. Twelve tablets of each brand were studied to obtain statistically significant result [9]. Results are shown in and figures ((5) to (7).

Results

Table (1): Diameter, Thickness , Hardness and Disintegration test results of the four brands of Amlodipine tablets:

Items	Average Diameter (mm)±SD	Average Thickness (mm)±SD	Average Hardness (Kg/cm2) ± SD	Average Disintegration (min:sec) ±SD	Content%
Brand A	10±0.01	3±0	5.95±0.45	0:12±0.1	100.1%
Brand B	9±0.02	3 ±0.01	5.83±0.59	0:12±0.1	99.5%
Brand C	8±0.02	3±0.002	5.97±0.44	0:12±0.1	99.5%
Brand D	8±0.01	2.5±0.02	6.0±0	0:30±0.1	98.5%

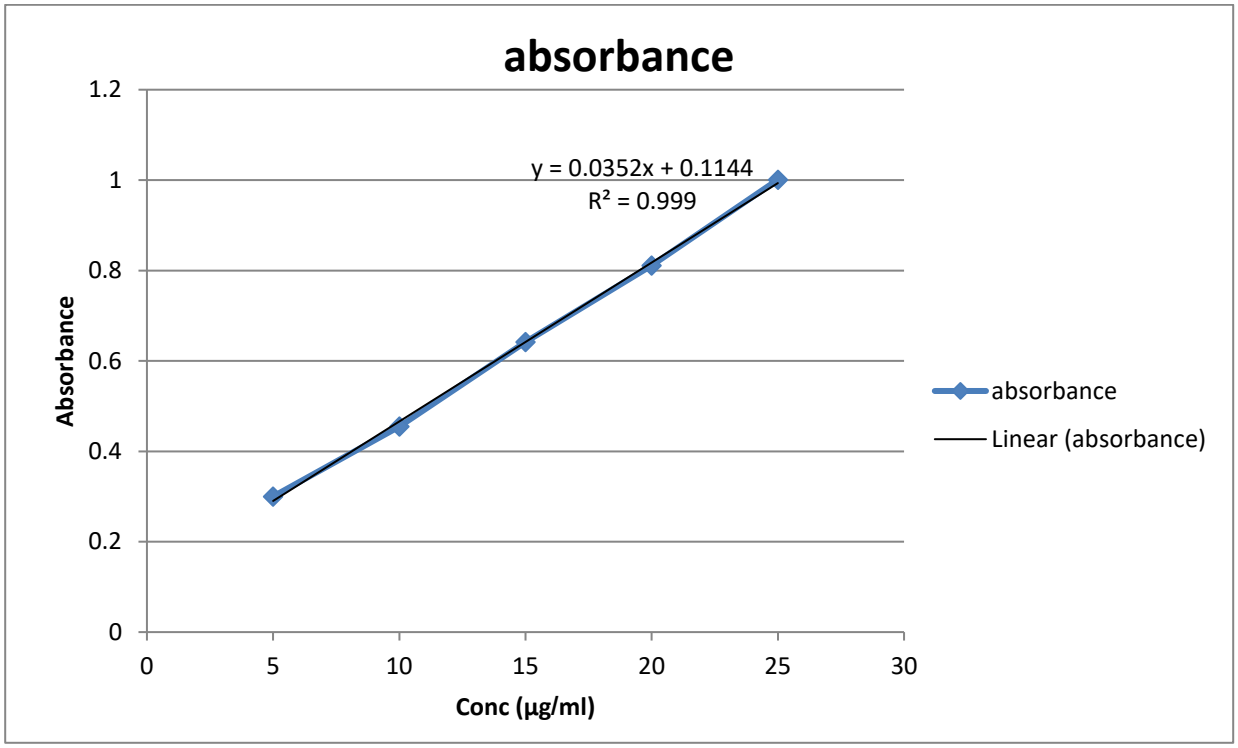

Figure (2): Amlodipine working standard calibration curve at HCL buffer solution pH 1.2

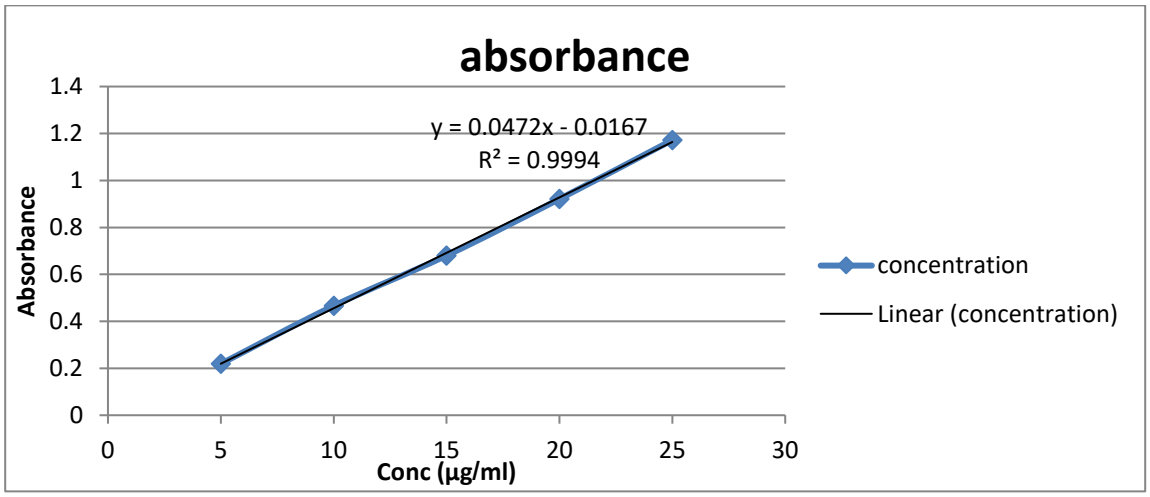

Figure (3): Amlodipine working standard calibration curve at Acetate buffer solution pH 4.6

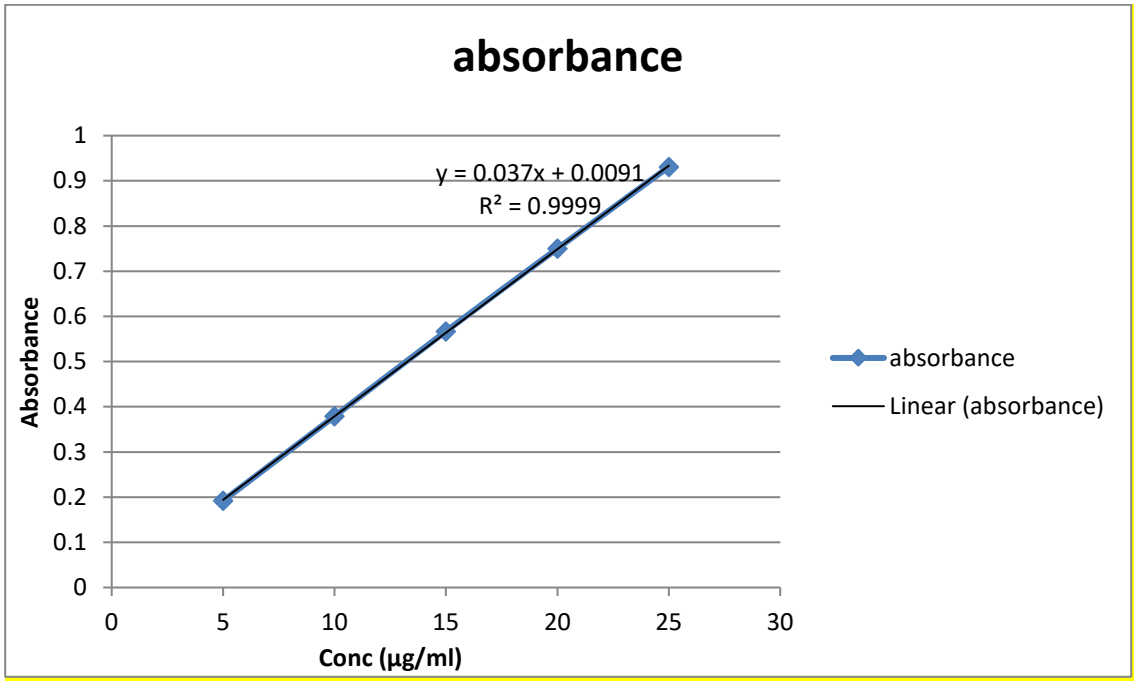

Figure (4): Amlodipine working standard calibration curve at Phosphate buffer solution pH 6.8

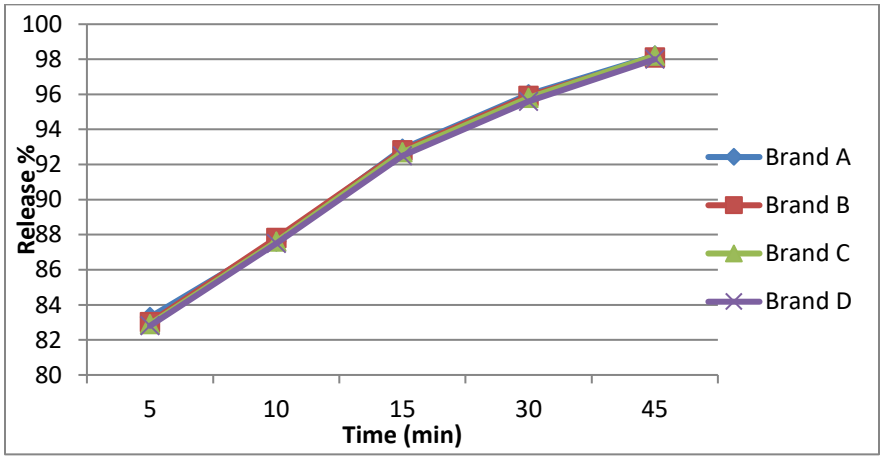

Figure (5): Dissolution profile of the four brands at HCL buffer solution pH 1.2 .

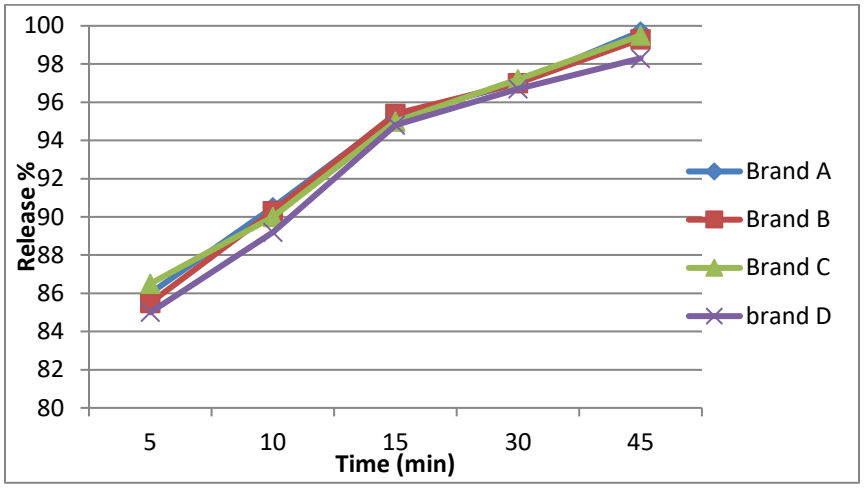

Figure (6): Dissolution profile of the four brands at Acetate buffer solution pH 4.5.

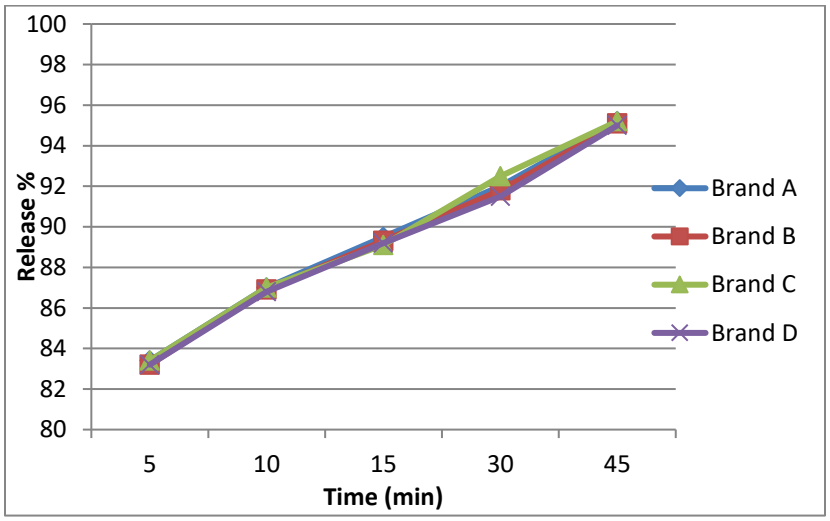

Figure (7): Dissolution profile of the four brands at Phosphate buffer solution pH 6.8.

Discussion:

Amlodipine besylate is a calcium channel blocker. In Sudan this drug is used widely to treat many diseases and there are many brands of it available in the market. In this study four brands of Amlodipine besylate 10 mg tablets were studied to collect information on possible interchangeability of different generic. Amlodipine besylate tablet brands were studied by using simple and cost effective in *vitro* dissolution method (Biowaiver).

In the present study the samples of three generics brands and innovator of Amlodipine besylate10mg tablets were collected randomly from the market, the three generics brands are locally manufactured (brand (B), brand (C) and brand (D)), the four brands were within their expiry dates and their

physicochemical properties and content percent were studied. The table shows that all the brands studied fulfill the specifications for diameter, thickness, hardness and disintegration. The four brands also pass the assay test as shown in the table. This means that the four brands are pharmaceutically equivalents.

Dissolution test was carried out for four brands to establish bioequivalence between the different brands. The test was carried out in three different buffer medias (pH1.2, pH4.5 and pH 6.8) to cover the whole GIT environment of different pH, and the percentage release in each point was calculated. Figures ((5) to (7)) show very rapid dissolution (85% or more of API is released in 15 min) in the three pH different buffer media. According to WHO requirements brand (B), brand (C) and brand (D) are bioequivalent and can be safely interchanged with innovator.

Conclusion

It can reasonably be concluded that, brand (B), brand (C) and brand (D) of Amlodipine besylate 10mg tablets are interchangeable with the innovator (brand A), because they meet the requirements of biowaiver according to WHO criteria.

Recommendations

Brand (B), brand (C) and brand (D) of Amlodipine besylate 10mg tablets are bioequivalent and can be interchanged safely with innovator. Further study should be conducted for other brands marketed in Sudan to ensure the possible interchangeability with the innovator.

The calibration curves for the three buffers were validated for linearity, however, they require further validation for more results reliability.

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