IJPSR (2013), Vol. 4, Issue 10 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 24 May, 2013; received in revised form, 30 July, 2013; accepted, 24 September, 2013; published 01 October, 2013

IN VITRO STUDIES OF AMLODIPINE BESYLATE TABLET AND COMPARISON WITH FOREIGN BRAND LEADER IN NEPAL

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Keywords:

Amlodipine besylate, *In vitro* studies, Biowaiver testing, Patient compliance, Biopharmaceutical classification system

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ABSTRACT: The aim of this study was to investigate the physicochemical properties of amlodipine besylate tablets. Tablets containing 5 mg of amlodipine besylate were prepared using Generally Regarded as Safe (GRAS) excipients. Preformulation studies were carried out using Fourier Transform Infrared (FTIR) spectroscopy and High Performance Liquid Chromatography (HPLC). Dissolution studies were performed to assess biowaiver criteria for Biopharmaceutical Classification System (BCS) Class I drug. Both the test and reference products conformed to pharmacopoeial requirements for physical parameter of tablets. FTIR studies revealed that there was no interaction between drug and excipients used in the test product. The percentage of drug remaining in the samples, subjected to the accelerated condition, complied the range specified in the United States Pharmacopeia (USP) monograph of amlodipine besylate. The dissolution of both the products was found to be more than 85 % in pH 1.2 buffer at the interval of 15 min. However, in pH 4.5 and pH 6.8 buffer media, only test product released more than 85 % drug, whereas the reference product did not. Mathematical analysis suggested that the release profiles obtained from the test and reference products in pH 4.5 and pH 6.8 buffer media were dissimilar. The finding of this study suggests that, under the tested conditions, the test product is better than the leading foreign brand available in Nepal. Prescribing pattern could be tailored based on the pharmaceutical quality of the product as well as cost of therapy to ensure better pharmacotherapy and patient compliance

INTRODUCTION: Cardiovascular diseases are increasing rapidly in the developing world [1]. Hypertension is one among the most important modifiable risk factors for cardiovascular disease [2], it affects approximately one billion people in the worldwide [3]. Treatment of high blood pressure can reduce cardiovascular mortality and morbidity [4].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.4(10).3958-64

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.4(10).3958-64

Amlodipine is a calcium channel blocker belonging to the dihydropyridine group, used in hypertension and prophylaxis of chronic stable angina pectoris [5, 6]. Various studies have shown a significant use and prescription of the amlodipine in cardiovascular disease.

It was reported that the most commonly prescribed drugs to cardiovascular patients in the internal medicine ward of Manipal Teaching hospital, Nepal was amlodipine [7], in which 34.88 % (n=45) were amlodipine and the most common cardiovascular disease condition among the patient was hypertension [7].

Similarly, in another study it was reported that 67% of total drugs for hypertension was calcium channel blocker, 97.14% was amlodipine and the rest 2.86 % was nifedipine [3]. Being the most popular drug among the calcium channel blocker, it need a special surveillance that assure the high quality of drugs ultimately help to enhance the quality of life in developing countries.

Amlodipine is slowly and almost completely absorbed from the gastrointestinal tract [5, 6]. Peak plasma concentrations are reached at 6-9 h of post dose [8, 9]. Amlodipine is extensively metabolized in the liver, but there is no significant pre-systemic or first pass metabolism and is slowly cleared with a terminal elimination half-life of 40-60 h [6, 9].Volume of distribution is large (21 L/kg), and there is a high degree of protein binding (98 %) [6]. Because of less extensive and less variable first pass metabolism, its oral bioavailability is higher (60% - 80%), and more consistent[7, 9]. Thus, an oral dosage form is preferable.

BCS classification helps to ensure clinical performance of immediate release dosage form by *in vitro* dissolution rather than empirical in human *in vivo* studies [10]. Amlodipine besylate is classified as BCS I drug [11]. BCS I drugs are those which are highly soluble and highly permeable [12]. A drug substance is considered highly soluble when the highest dose strength is soluble in 250 ml or less of aqueous media over the pH range of 1.2-6.8 [11, 13]. And a drug substance is considered to be highly permeable when the extent of absorption in humans is determined to be 90% or more of an administered dose based on a mass balance determination or in comparison to an intravenous reference dose [11].

Amlodipine is listed in World Health Organization (WHO) model list of essential medicines as antihypertensive medicine in 5 mg tablet [11]. Amlodipine is slightly soluble in water, freely soluble in methanol, sparingly soluble in anhydrous ethanol, slightly soluble in 2-propanol [14]. Nepal has marketing authorization for amlodipine as an immediate release dosage form in the strength 2.5 mg, 5 mg and 10 mg [15]. It was found that the aqueous solubility of the amlodipine besylate in distilled water was 2.6 mg/ml [16]. Therefore, the highest dose 10 mg amlodipine dissolves in less than 250 ml of different buffers pH 1.2, pH 4.5 and pH 6.8. Thus, amlodipine is a highly soluble drug according to WHO (D/S ratio ≤ 250 ml). Permeability of amlodipine besylate is classified "high" due to metabolic excretion in urine (90-95%) [17].

Most of pharmaceuticals dosage form is administered through the oral route, so it is important to know how these materials behave during their passage through the gastrointestinal tract. Small intestine is the major site of drug absorption and environment with the lumen of the gastrointestinal tract has a major effect on the rate and extent of drug absorption [18]. According to the pH- partition hypothesis theory, the epithelial acts as a lipid barrier towards the drugs.

The unionized form of weakly acidic or basic drugs will pass the gastrointestinal epithelia. A weakly acidic drug is more likely to be absorbed from the stomach where it is unionized and weakly basic drug is to be absorbed from the intestine [18]. The pKa of amlodipne besylate is about 8.6 at 25^{o}C [4] . Bases in the pKa range 5-10 are generally affected by changes in pH and hence their absorption is pH dependent [19].

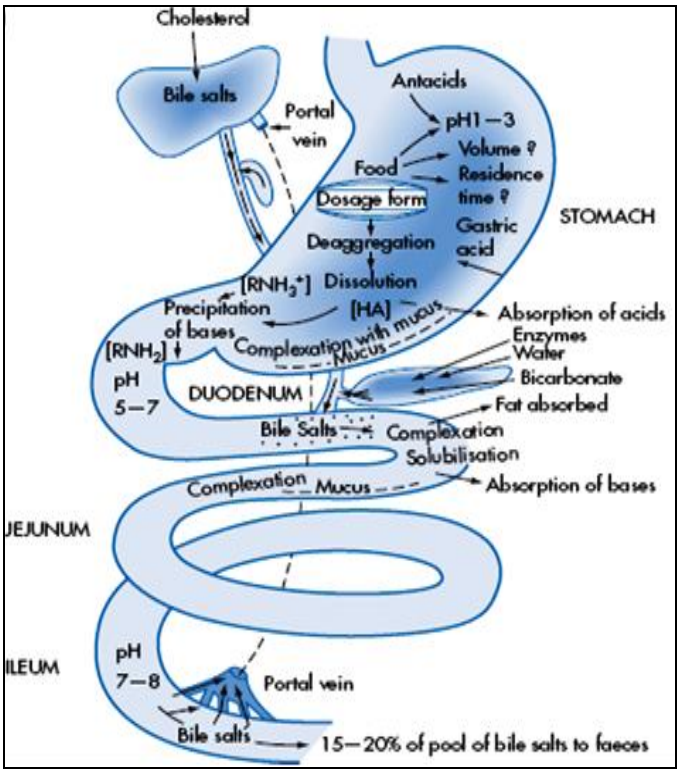

FIG. 1: REPRESENTATION OF THE PROCESSES OCCURING ALONG THE GI TRACT, AND OF THE FACTORS THAT MUST BE TAKEN INTO ACCOUNT IN CONSIDERING DRUG ABSORPTION (REPRODUCED FROM REF: 20)

The *in vitro* analysis in different pH medium provides better insight into the absorption process. Therefore, the aim of this study was to investigate *in vitro* studies of amlodipine besylate tablet (test product) and comparison with foreign brand leader in Nepal (reference product).

MATERIALS AND METHODS:

Materials: Amlodipine besylate was procured from Cadilla Pharmaceuticals (Ahmedabad, India). Microcrystalline cellulose was bought from FMC International (Wallingstown, Ireland). Calcium hydrogen phosphate was obtained from Hindustan Phosphate (Indore, India). Sodium starch glycolate was purchased from Maruti Chemicals (Rakanpur, India). Brilliant blue and quinoline yellow were procured from Roha Dyechem (Mumbai, India). Colloidal anhydrous silica was bought from Evonnik Industries (Untevekanalstr, Germany). Magnesium stearate was purchased from Nikita Chemicals (Nagpur, India). All the reagents of analytical grades were used without further purification.

Methods:

Preformulation studies:

Qualitative: Binary blend (1:1 w/w) were prepared by physically mixing amlodipine besylate with microcrystalline cellulose, calcium hydrogen phosphate, sodium starch glycolate , brilliant blue , quinoline yellow, colloidal anhydrous silica and magnesium stearate . Spectroscopic analysis was carried out in FTIR system (IR Prestige-21, Shimadzu, Japan).

Quantitative*:* Samples were prepared by mixing drug and excipients in presence of 25% moisture [21]. The blend was stored in an oven at 100^{o}C for 3 h [21]. Samples were suitably diluted in the mobile phase, filtered through membrane filter (Ultipor®, 0.2 µm, Pall India, India) and analyzed in HPLC (LC2010 AHT, Shimadzu, Japan).

The system consisted of a UV detector, reverse phase C_{18} column (4.6 x 150 mm, Protecol, SGE Analytical Science, Australia), a pump, and an automatic injector. The method previously described was slightly modified [22]. Briefly, the wavelength of the UV visible detector was 237 nm, the column temperature was maintained at 30^{o}C, the flow rate was 1mL/min, and injection volume was 50 µL. The mobile phase consisted of methanol: acetonitrile: pH 3 buffer (7:3:10).

Physical parameters: Physical parameters like shape, size, color, hardness, uniformity of weight, friability, disintegration time (DT), and pH were evaluated using standard pharmacopoeial method [14].

Dissolution: Studies were performed using USP Apparatus II (TDT-082, Electrolab, Mumbai, India) at 75 rpm with 500 ml buffer medium. USP buffer solutions of pH 1.2 (hydrochloric acid), pH 4.5 (acetate), and pH 6.8 (phosphate) were maintained at 37 ± 0.5^{o}C. Amlodipine besylate was assayed using HPLC method as described above. Similarly solubility studied was carried out as previously described with slightly modification [16].

Statistical analysis: The dissolution profiles of the test and reference products were compared using similarity factor (f_2). An f_2 value of 50 or greater (50-100) reflected equivalence and lower values denoted dissimilar profiles [11, 13].

Following equation was used to calculate f_2:

$$f_2 = 50 \log \{[1 + 1/n\Sigma^{n=1}(R_t - T_t)^2]^{-0.5} \times 100\}$$

Where, T_t and R_t are percent drug dissolved at each time point for test and reference product, respectively.

RESULTS AND DISCUSSION:

Physical properties: Tablets require a certain amount of strength, or hardness and resistance to friability, to withstand mechanical shocks during handling in manufacture, packing, shipping and handling of retailers by patients. To avoid the problems that may arise during packaging transportation or crumble in the hands patients, hardness of 4 kg/cm^2 and friability not more than 1% w/w considered suitable for handling the tablets [23]. Results from both the products complied **(Table 1)**.

TABLE 1: SUMMARY OF PHYSICAL PARAMETERS

Parameters	Test Product	Reference Product
Packaging	Opaque blister	Glass clear blister
Shape	Round, biconvex	Round, flat
Color	Green	White
Diameter (mm)	6.57 ± 0.01 (n=10)	8.31 ± 0.01 (n=10)
Thickness(mm)	3.32 ± 0.03 (n=10)	3.23 ± 0.06 (n=10)
Hardness (kg/cm^2)	7.30 ± 0.07 (n=10)	3.92 ± 0.55 (n=10)
Friability	0.00 %	0.04 %
Surface pH	7.28	6.82
DT (min)	1 (n=6)	3 (n=6)
Wt (mg)	110 ± 1 (n=20)	225 ± 2 (n=20)

Compatibility studies: Preformulation is the first step in the rational formulation of an active pharmaceutical ingredient. It is an investigation of the physical-chemical properties of the drug substance, alone and in combination with excipients. Assessment of possible incompatibilities between the drug and different excipient is an important part of preformulation [24]. FTIR spectra of amlodipine besylate showed characteristic amine stretching and bending at 3167.47 cm^{-1} and 1697.36 cm^{-1}, respectively **(Fig. 2, Fig. 3)**. The peak observed at 3167.47 cm^{-1} can be attributed to N^+ - H stretching mode and the sharp band at 1697cm^{-1} is due to C=O stretching vibration [25]. The peaks were unaltered in the samples containing binary mixtures of drug and excipients. This suggests that there is no incompatibility between drug and excipients.

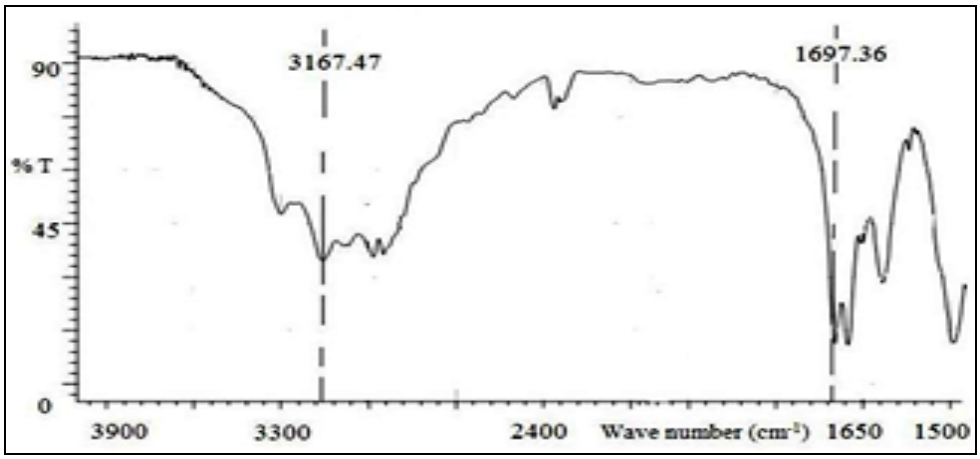

FIG. 2: FTIR SPECTRA OF AMLODIPINE BESYLATE

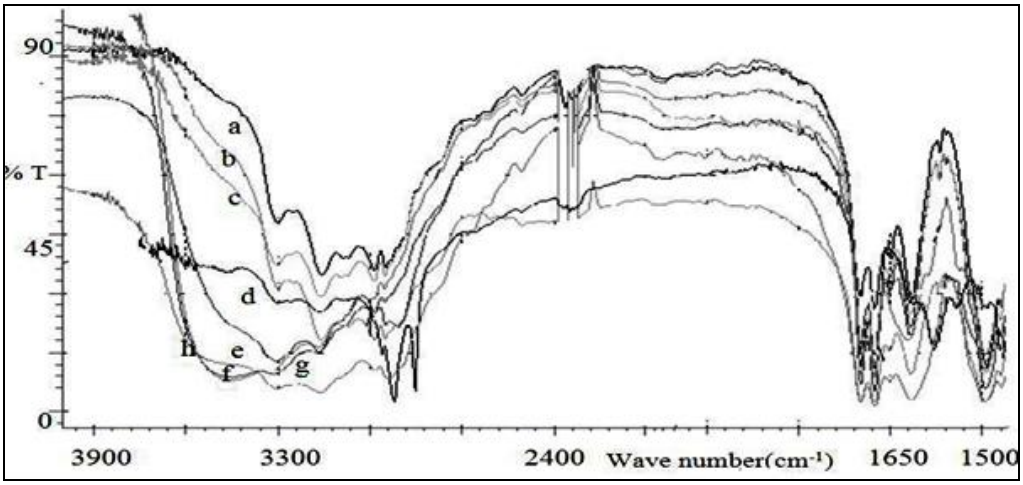

FIG. 3: FTIR SPECTRA OF; (a) Amlodipine Besylate (b) Amlodipine Besylate + Calcium Hydrogen Phosphate (c) Amlodipine Besylate + Colloidal Anhydrous Silica (d) Amlodipine Besylate + Microcrystalline Cellulose (e) Amlodipine Besylate + Sodium Starch Glycolate (f) Amlodipine Besylate + Magnesium Stearate (g) Amlodipine Besylate + Brilliant Blue (h) Amlodipine Besylate + Quinoline Yellow

Other studies have also reported compatibility of amlodipine besylate with excipients like sodium starch glycolate, microcrystalline cellulose, colloidal silicon dioxide and camphor [26]. Furthermore, quantitative studies were performed in HPLC to assess interaction of amlodipine with the excipients, if any.

The amount of amlodipine besylate remaining in the samples subjected to accelerated condition, in presence of excipients, was determined. The result complied with the limit of assay specified in the United States Pharmacopeia (**Table 2**).

TABLE 2: COMPATIBILITY OF AMLODIPINE BESYLATE WITH DIFFERENT EXCIPIENTS

Binary mixture with	Amlodipine besylate remaining (%)
Drug control	98.94 ± 0.59
Microcrystalline cellulose	99.16 ± 1.12
Calcium hydrogen phosphate	98.42 ± 2.73
Brilliant blue	101.65 ± 0.13
Quinoline yellow	101.92 ± 0.59
Colloidal anhydrous silica	101.65 ± 0.13
Magnesium stearate	91.51 ± 0.5
Sodium starch glycolate	93.90 ± 0.69
Values are expressed as mean ±	Standard deviation (n=3).

In the case of binary mixture with magnesium stearate and amlodipine besylate, the remaining quantity of amlodipine was found to meet the pharmacopoeial limit in marginal range. However, in the tablet formulation the ratio of lubricant is very low, which may not impose any incompatibility issue. Furthermore, the remaining amount of amlodipine besylate in the test product subjected to stress testing was 96.33%.

Hence, it can be inferred that all the excipients used to formulate tablet are compatible with amlodipine besylate. Drug-excipients compatibility study helps to formulate safe and efficient dosage form [21]. Moreover, careful selection of excipient can help to achieve equivalence with the innovator product [27].

Dissolution studies: Release study was conducted in pH 1.2, pH 4.5 and pH 6.8 buffer media. In pH 1.2 buffer medium, both the products released more than 85% drug at 15 min **(Fig. 4)**.

However, in pH 4.5 and pH 6.8 buffer media, marked difference in dissolution was observed **(Fig. 5, 6)**.

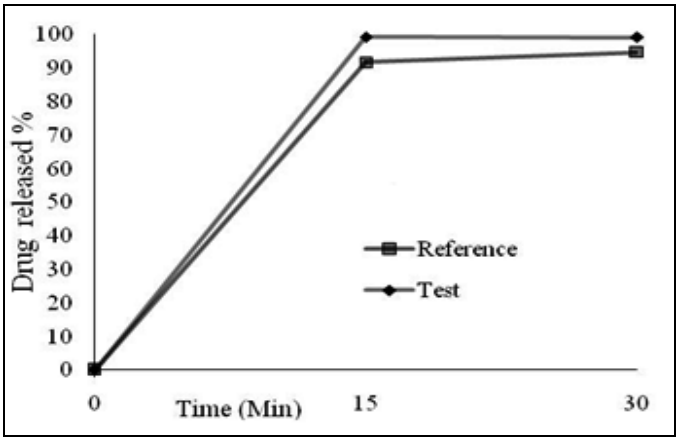

FIG. 4: DISSOLUTION PROFILES OF TEST AND REFERENCE PRODUCTS IN pH 1.2 BUFFER

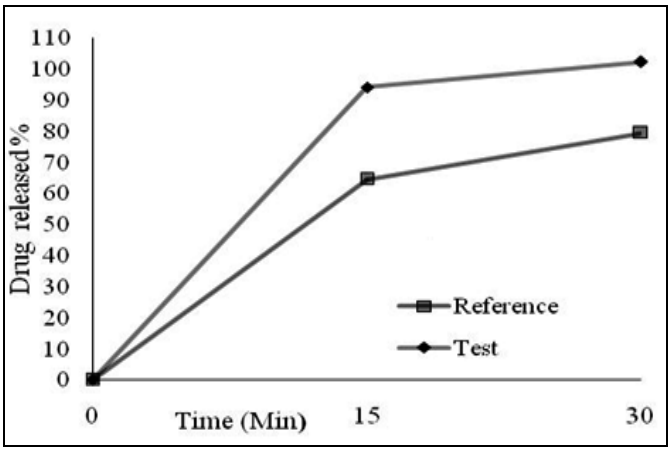

FIG. 5: DISSOLUTION PROFILES OF TEST AND REFERENCE PRODUCTS IN pH 4.5 BUFFER

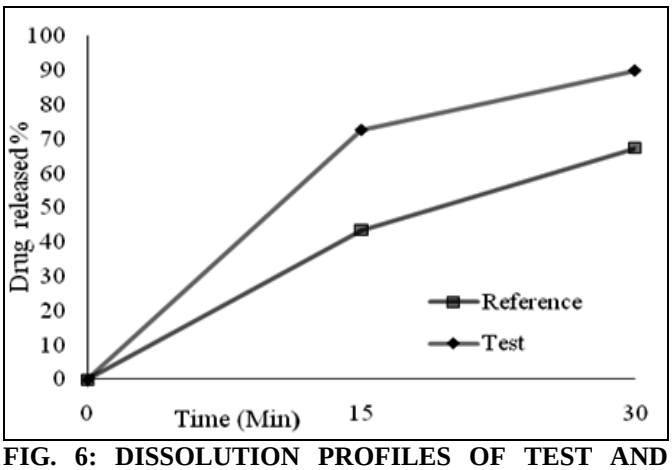

FIG. 6: DISSOLUTION PROFILES OF TEST AND REFERENCE PRODUCTS IN pH 6.8 BUFFER

The test product released more than 85% drug at 30 min but the reference product did not. Solubility studies were conducted in the dissolution media. It was found that the solubility of amlodipine besylate decreased as the pH of medium increased from 1.2 to 6.8.

The solubility observed for amlodipine besylate was 0.38 mg/mL ± 0.017, 0.31 mg/mL ± 0.005 and 0.11mg/mL ± 0.002 in pH 1.2, pH 4.5 and pH 6.8 buffers, respectively.

Furthermore, dissolution profile comparison was carried out using similarity factor (f_2), the similarity factor was found to be 31.92 and 32.32 in pH 4.5 and pH 6.8 buffers, respectively. The dissolution profiles would have been similar for the values of $f_2 > 50$ [11]. Thus, the dissolution profiles of test and reference products were dissimilar in pH 4.5 and pH6.8 buffer media. According to WHO, to meet the biowaiver condition for BCS Class I active pharmaceutical ingredient **(**API), more than 85 % dissolution of the labeled amount is required within 30 min in standard media at pH 1.2, 4.5 and 6.8 using the paddle apparatus at 75 rpm or the basket apparatus at 100 rpm [8].

If within 15 minutes, more than 85% of the API is released from the comparator and the multisource formulation under the above-mentioned conditions, the products will be considered very rapidly dissolving. In this case the products are deemed to be equivalent and a profile comparison is not required [11]. Several comparison studies are reported for amlodipine products. In some cases, comparator product were found to be equivalent to the innovator product [17], whereas in the other not [27]. Hence, careful selection of the excipients and the manufacturing process has profound importance in formulating dosage form for amlodipine besylate.

CONCLUSION: The test product complied the biowaiver condition of dissolution for BCS I drugs whereas the reference product did not. Physical properties of the test product, especially in terms of hardness and friability, were found to be better. Moreover, the test product is almost half times cheaper than the reference product. Considering the better quality and lower cost, present study suggests that the test product is more likely to improve the therapeutic benefit and patient compliance.

ACKNOWLEDGEMENT: We express our sincere gratitude to Hari Bhakta Sharma-Executive Director, Mani Ratna Shakya-Vice President (Technical affairs), Damodar Gautam-R&D Manager and Management authorities of Deurali Janta Pharmaceuticals Pvt. Ltd for their support and encouragement towards this research.

REFERENCES:

1. Reddy KS and Yusuf S: Emerging epidemic of cardiovascular disease in developing countries. Circular 1998; 97:596-601.
2. Singh RB, Suh IL, Singh VP, Chaithiraphan S, Laothavorn P, Sy RG, Babilonia NA, Rahman AR, Sheikh S, Tomlinson B and Sarraf N: Hypertension and stroke in Asia- Prevalence, control and strategies in developing countries for prevetion. J Hum Hypertens 2000; 14(10-11):749-763.
3. Joshi M, Rao BS and Khan GM: Study of drug use in essential hypertension and their compliance. Kathmandu University Journal of Science, Engineering & Technology 2006; 2(1):1-14.
4. Alderman MH and Wagner EH: Blood pressure control to reduce cardiovascular morbidity and mortality- Today and Tomorrow. Effective clinical practice 1998; 1:23-25.
5. Reid JL, Meredith PA, Donnelly R and Elliot HL: Pharmacokinetics of Calcium Antagonists. Journal of Cardiovascular Pharmacology 1988; 12(suppl.7):S22-S26.
6. Meredith PA and Elliott HL: Clinical Pharmacokinetics of Amlodipine. Clin Pharmacokinet 1992; 22(1):22-31.
7. Shankar PR, Partha P and Shenoy P: Prescribing pattern of drugs among patient admitted with cardiovascular disorders in the internal medicine ward: Prescribing pattern in inpatients. Internet Journal of Pharmacology 2002; 1(2):1-5.
8. Tripathi KD: Essentials of Medical Pharmacology. Jay Pee Brothers Medical Publishers, New Delhi, Fifth Edition, 2003:496-497.
9. Abernethy DR: Pharmacokinetics and pharmacodynamics of amlodipine. Cardiology 1992; 80 (suppl. 1):31-6.
10. Dahan A, Miller JM and Amidon GL: Prediction of solubility and permeability class membership- Provisional BCS classification of the world's top oral drugs. AAPS J 2009; 11(4):740-6.
11. WHO Expert committee on specifications for Pharmaceutical Preparations-WHO Technical Report Series, Annex 8: Proposal to waive *in vivo* bioequivalence requirements for World Health Organization model list of essential medicines immediate-release, solid oral dosage forms. World Health Organization, Geneva, 2006.
12. Amidon GL, Lennernas H, Shah VP and Crison JR: A theoretical basis for a biopharmaceutic drug classification: the correlation of in vitro drug product dissolution and *in vivo* bioavailability. Pharm Res 1995; 12; 413.
13. Guidance for industry: Waiver of *in vivo* bioavailability and bioequivalence studies for immediate release dosage form based on a biopharmaceutical classification system. Centre for Drug Evaluation and Research, Rockville, 2000.
14. British Pharmacopoeia. British Pharmacopeia Commission, London, Vol. I, 2010.
15. List of Manufacture with product(s) including ingredient. Department of Drug Administration, Nepal:1-90. http://www.dda.gov.np/registered_drugs/total%20drug%20list%20with%20ingredient%20and%20price.pdf
16. Dahima R, Pachori A and Netam S: Formulation and evaluation of mouth dissolving tablet containing amlodipine besylate solid dispersion. Int J Chem Tech Res. 2010, 2(1):706-715.
17. Ramenskaya GV, Shohin IE, Vasilenko GF and Malashenko EA: *In vitro* dissolution kinetics of

amlodipine tablets marketed in Russia under biowaiver condition. Dissolution Technologies 2010:20-2.
18. Aulton ME: The Science of Dosage Form – Pharmaceutics. Churcil Livingstone, London, Second Edition 2002: 222-242.
19. Bramahnkar DM and Jaiswal SB: Biopharmaceutics BIopharmaceutics and Pharmacokinetics- A Treatise. Vallabh Prakhashan, New Delhi, First Edition 1995: 34.
20. Florescence AT and Attwood D: Physiochemical Principles of Pharmacy. Pharmaceutical Press, London, Fourth Edition 2006: 342.
21. Abodh A, Omari AL, Badwan AA and Jaber AMY: Amlodipine besylate excipients interaction in solid dosage form. Pharm Dev Technol 2004; 9(1):15-24.
22. United States Pharmacopeia and National Formulary. Rockville, Twenty ninth Edition, Vol. II, 2011.
23. Thapa HS and Jali R: Formulation and evaluation of release kinetics of ketorolac tromethamine from matrix tablets. JNPA 2008; 25(1):17-33.
24. Bharate SS, Bharate SB and Bajaj AN: Interactions and incompatibilities of pharmaceutical excipients with active pharmaceutical ingredients- A comprehensive review. J. Excipients and Food Chem 2010; 1(3).
25. Rahman N and Narsul HMd: Validated spectroscopic methods for determining methods for the determination of amlodipine besylate in drug formulations using 2, 3-dichloro 5, 6-dicyano 1, 4- bezoquinone and ascorbic acid. J Pharm Biomed Anal 2003; 31: 381-392.
26. Narmada Gy, Mohini K, Prakash RB, Gowrinath DXP and Kumar KS: Formulation, evaluation and optimization of fast dissolving tablets containing amlodipine besylate by sublimation method. Ars Pharm 2009; 50(3): 129-144.
27. Olusola AM, Olubukola OO, Emeka OH and Lillian AE: Equivalence of two generic brands of amlodipine besylate under biowaiver conditions. J Pharm Pharm Sci 2012; 4(2):256-8.

How to cite this article:
Pant T, Mishra K and Subedi RK: *In vitro* studies of Amlodipine besylate tablet and comparison with foreign brand leader in Nepal. *Int J Pharm Sci Res* 2013: 4(10); 3958-3964. doi: 10.13040/IJPSR. 0975-8232.4(10).3958-64